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(54) Title: SYNTHETIC PEPTIDES HAVING PITUITARY GROWTH HORMONE RELEASING ACTIVITY (57) Abstract Novel peptides having the amino acid sequence selected from a group consisting of <div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> $\begin{array}{c} X_1 \\ \downarrow \\ (X_2) a - A_1 - A_2 - A_3 - A_4 - A_5 - Y(I) \\ \downarrow \\ (X_3) b \end{array}$ </div> <div style="text-align: center;"> $\begin{array}{c} X'_1 \\ \downarrow \\ (X'_2) a - A_1 - A_2 - A_3 - A_4 - A_5 - A_6 - Y(II) \\ \downarrow \\ (X'_3) b \end{array}$ </div> </div> <p>. In addition, this invention encompasses a combination of compounds comprising (a) at least one peptide having the for- mula III</p> <div style="text-align: center;"> $\begin{array}{c} X''_1 \\ \downarrow \\ (X''_2) a - A_1 - A_2 - A_3 - A_4 - A_5 - A_6 - Y(III) \\ \downarrow \\ (X''_3) b \end{array}$ </div> <p>and (b) at least one growth promoting agent. These peptides and combinations act directly on the pituitary to release growth hormone therefrom.</p>		

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SYNTHETIC PEPTIDES HAVING
PITUITARY GROWTH HORMONE RELEASING ACTIVITY

Background of the Invention

1. Field of the Invention

This invention relates to peptides which possess pituitary growth hormone releasing activity and to combinations comprising at least one peptide and at least one growth promoting agent, which combination possesses synergistic pituitary growth hormone releasing activity.

2. Description of the Prior Art

Growth hormone, which is secreted from the pituitary, causes growth of all tissues of the body that are capable of growing. In addition, growth hormone is known to have the following basic effects on the metabolic process of the body:

1. Increased rate of protein synthesis in all cells of the body;
2. Decreased rate of carbohydrate utilization in cells of the body;
3. Increased mobilization of free fatty acids and use of fatty acids for energy.

A deficiency in growth hormone secretion can result in various medical disorders, such as some instances of dwarfism.

Various ways are known to release growth hormone. For example, chemicals such as arginine, L-3,4-dihydroxyphenylalanine (L-DOPA), glucagon, vasopressin, and insulin induced hypoglycemia, as well as activities such as sleep and exercise, indirectly cause growth hormone to be released from the pituitary by acting in some fashion on the hypothalamus perhaps either to decrease



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somatostatin secretion or to increase an unknown endogenous growth hormone-releasing hormone or both.

Compounds which directly act on the pituitary to release growth hormone include prostaglandin E₁ and E₂, theophylline, and cyclic nucleotides. However, these compounds neither specifically release growth hormone nor are they believed to act at the putative growth hormone-releasing hormone receptors in the peripheral membrane of the pituitary cell to initiate growth hormone release.

In addition, under special conditions certain chemically defined peptides, e.g., vasopressin, thyrotropin-releasing hormone (TRH), luteinizing hormone-releasing hormone (LH-RH), α -melanocyte-stimulating hormone (α -MSH), glucagon, substance P, neurotensin; Met-enkephalin, β -endorphin, chlorea-enterotoxin, and basic myelin protein, act to release growth hormone from the pituitary. However, only TRH acts directly on the pituitary to elicit this response. Furthermore, the above listed peptides release other pituitary hormones and under most experimental conditions do not release growth hormone. For example, TRH does not release growth hormone in normal rats or in normal humans or from pituitaries of normal rats or monkeys. In vitro, TRH releases growth hormone, prolactin, and thyroid stimulating hormone (TSH) in certain species, and, in vivo, TRH releases these hormones from bovine pituitary.

Vasopressin's induced release of growth hormone is considered to be due to a non-specific response to stress caused by administration of high dosages of vasopressin.

Accordingly it would be highly desirable to have a compound or combination of compounds which



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directly acts on the pituitary under normal experimental conditions to effect the release of growth hormone therefrom. Such compound or combination of compounds would be useful in vitro, e.g., as unique research tools for understanding how growth hormone secretion is regulated at the pituitary level and would also be useful in vivo, e.g., to treat symptoms related to growth hormone deficiencies, to increase the rate and extent of growth in commercial animals, to increase milk yield in commercial animals, and to reduce the number of mucosal erosions induced by hypoxemia.

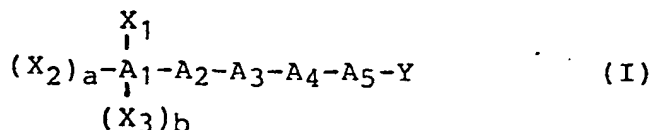
Summary of the Invention

In accordance with the present invention there is provided peptides which act directly on the pituitary under normal experimental conditions in vitro to release growth hormone therefrom. In addition, there is provided a combination of compounds which act directly on the pituitary under normal experimental conditions in vitro to synergistically release growth hormone therefrom.

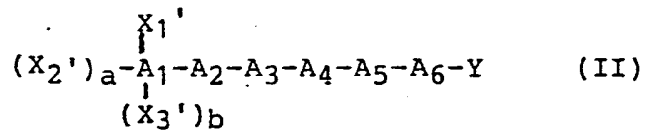
These growth hormone releasing compounds and combinations can be utilized in vitro as unique research tools for understanding, inter alia, how growth hormone secretion is regulated at the pituitary level.

Also, the growth hormone releasing peptides of the instant invention can also be administered in vivo to increase growth hormone release.

More particularly, this invention encompasses novel peptides having the formulas I and II



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wherein X_1 , X_2 , X_3 , and X_1' , X_2' , and X_3' are selected from a group consisting of N-terminal and desamino alpha-carbon substitutions; a and b are 0 or 1, provided that a and b are always 0 when A_1 is a desamino residue; A_1 and A_4 are selected from a group consisting of histidyl, arginyl, lysyl, α -naphthylalanyl, β -naphthylalanyl, isoquinolyl, tyrosyl, tryptophyl, phenylalanyl, homologues and analogues thereof, and, with respect to A_1 only, the desamino forms thereof; A_2 and A_5 are selected from a group consisting of D-histidyl, D-arginyl, D-lysyl, D- α -naphthylalanyl, D- β -naphthylalanyl, D-isoquinolyl, D-tyrosyl, D-tryptophyl, D-phenylalanyl, homologues and analogues thereof, and, with respect to A_5 only, the descarboxy forms thereof; A_3 is selected from a group consisting of glycyl, alanyl, valyl, leucyl, isoleucyl, prolyl, seryl, threonyl, methionyl, aspartyl, glutamyl, asparaginyl, glutaminyl, histidyl, D-alanyl, D-valyl, D-leucyl, D-isoleucyl, D-prolyl, D-seryl, D-threonyl, D-methionyl, D-aspartyl, D-glutamyl, D-asparaginyl, D-glutaminyl, D-histidyl, and homologues and analogues thereof; A_6 is selected from a group consisting of amino acid residues of the L- and D-configuration, homologues and analogues thereof, and the descarboxy forms thereof; and Y is selected from a group consisting of C-terminal and descarboxy alpha-carbon substitutions; and the pharmaceutically acceptable salts thereof; provided that (a) when (1) is 1 and b is 0 and X_1 and X_2 are selected from the group consisting of tyrosyl, tryptophyl, and phenylalanyl; (3) A_3 is selected from the group consisting of glycyl, alanyl, valyl, leucyl, isoleucyl, prolyl, seryl, threonyl, methionyl, aspartyl, glutamyl,



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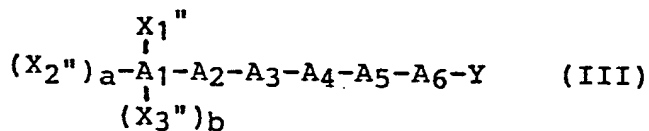
asparaginy, glutaminy, and histidy; and (4) Y is selected from the group consisting of $-NR_1R_2$, $-OR$, and $-CH_2OR$, wherein R, R_1 , and R_2 are selected from a group consisting of hydrogen and straight and branched chain alkyl groups containing 1-6 carbon atoms; then at least one of A_2 and A_5 is selected such that it is not from a group consisting of D-tyrosyl, D-tryptophyl, D-phenylalanyl, and, with respect to A_5 , the descarboxy forms thereof; (b) when (1) a is 1 and b is 0 and X_1 and X_2 are selected from the group consisting of $-H$ and $-CH_3$; (2) A_2 and A_5 are selected from the group consisting of D-tyrosyl, D-tryptophyl, D-phenylalanyl, and, with respect to A_5 , the descarboxy forms thereof; (3) A_3 is selected from the group consisting of glycyl, alanyl, valyl, leucyl, isoleucyl, prolyl, seryl, threonyl, methionyl, aspartyl, glutamyl, asparaginy, glutaminy and histidy; and (4) Y is selected from the group consisting of $-NR_1R_2$, $-OR$, and $-CH_2OR$; wherein R, R_1 , and R_2 are selected from a group consisting of hydrogen and straight and branched chain alkyl groups containing 1-6 carbon atoms; then at least one of A_1 and A_4 is selected such that it is not from a group-consisting of tyrosyl, tryptophyl, and phenylalanyl; (c) when (1) a is 1 and b is 0 and X_1' and X_2' are selected from the group consisting of $-H$, $-CH_3$, and $-CHOCH_3$; (2) A_1 and A_4 are selected from the group consisting of tyrosyl, tryptophyl, and phenylalanyl; (3) A_3 is selected from the group consisting of glycyl, alanyl, valyl, leucyl, isoleucyl, seryl, threonyl, methionyl, asparaginy, and glutaminy; (4) A_6 is selected from the group consisting of asparaginy, glutaminy, glutamyl, arginy, lysyl, seryl, threonyl, and the descarboxy forms thereof; and (5) Y is selected from the group consisting of $-NR_1R_2$, $-OR$, and $-CH_2OR$, wherein R, R_1 , and R_2 are selected from a group consisting of hydrogen and straight and branched chain alkyl groups containing 1-6 carbon atoms; then at



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least one of A₂ and A₅ is selected such that it is not from a group consisting of D-tyrosyl, D-tryptophyl, and D-phenylalanyl; and (d) when (1) a is 1 and b is 0 and X₁' and X₂' are selected from the group consisting of -H, -CH₃, and -CHOCH₃; (2) A₂ and A₅ are selected from the group consisting of D-tyrosyl, D-tryptophyl, and D-phenylalanyl; (3) A₃ is selected from the group consisting of glycyl, alanyl, valyl, leucyl, isoleucyl, seryl, threonyl, methionyl, asparaginy, and glutaminy; (4) A₆ is selected from the group consisting of asparaginy, glutaminy, glutamyl, arginy, lysyl, seryl, threonyl, and the descarboxy forms thereof; and (5) Y is selected from the group consisting of -NR₁R₂, -OR, and -CH₂OR, wherein R, R₁, and R₂ are selected from a group consisting of hydrogen and straight and branched chain alkyl groups containing 1-6 carbon atoms; then at least one of A₁ and A₄ is selected such that it is not from a group consisting of tyrosyl, tryptophyl, and phenylalanyl.

In addition, this invention encompasses a combination of compounds comprising (a) at least one peptide having the formula III



wherein X₁'', X₂'', and X₃'' are selected from a group consisting of N-terminal and desamino alpha-carbon substitutions; a and b are 0 or 1, provided that a and b are always 0 when A₁ is a desamino residue; A₁ and A₄ are selected from a group consisting of histidyl, arginy, lysyl, α-naphthylalanyl, β-naphthylalanyl, isoquinolyl, tyrosyl, tryptophyl, phenylalanyl, homologues and analogues thereof, and, with respect to A₁ only, the des-

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amino forms thereof; A₂ and A₅ are selected from a group consisting of D-histidyl, D-arginyl, D-lysyl, D- α -naphthylalanyl, D- β -naphthylalanyl, D-isoquinolyl, D-tyrosyl, D-tryptophyl, D-phenylalanyl, homologues and analogues thereof, and, with respect to A₅ only, the descarboxy forms thereof; A₃ is selected from a group consisting of glycyl, alanyl, valyl, leucyl, isoleucyl, prolyl, seryl, threonyl, methionyl, aspartyl, glutamyl, asparaginy, glutaminy, histidyl, D-alanyl, D-valyl, D-leucyl, D-isoleucyl, D-prolyl, D-seryl, D-threonyl, D-methionyl, D-aspartyl, D-glutamyl, D-asparaginy, D-glutaminy, D-histidyl, and homologues and analogues thereof; A₆ is selected from a group consisting of amino acid residues of the L- and D-configuration, homologues and analogues thereof, and the descarboxy forms thereof; and Y is selected from a group consisting of C-terminal and descarboxy alpha-carbon substitutions; and the pharmaceutically acceptable salts thereof; and (b) at least one growth promoting agent.

Detailed Description of the Preferred Embodiments

The peptides of this invention have the amino acid residue sequence represented by formulas I-III, supra.

All amino acid residues identified herein are in the natural or L-configuration unless otherwise specified.

Abbreviations for amino acid residues are used in accordance with the following standard peptide nomenclature:

Tyr	L-tyrosyl	Ile	L-isoleucyl
D-Tyr	D-tyrosyl	D-Ile	D-isoleucyl
Gly	glycyl	Leu	L-leucyl



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Phe	L-phenylalanyl	D-Leu	D-leucyl
D-Phe	D-phenylalanyl	Thr	L-threonyl
Met	L-methionyl	D-Thr	D-threonyl
D-Met	D-methionyl	Val	L-valyl
Ala	L-alanyl	D-Val	D-valyl
D-Ala	D-alanyl	Pro	L-prolyl
Ser	L-seryl	D-Pro	D-prolyl
D-Ser	D-seryl	Gln	L-glutaminyl
Lys	L-lysyl	D-Gln	D-glutaminyl
D-Lys	D-lysyl	Glu	L-glutamyl
Asn	L-asparaginy	D-Glu	D-glutamyl
D-Asn	D-asparaginy	Trp	L-tryptophyl
His	L-histidyl	D-Trp	D-tryptophyl
		L-Asp	L-aspartyl
D-His	D-histidyl	D-Asp	D-aspartyl
Cys	L-cysteinyl	Arg	L-arginyl
D-Cys	D-cysteinyl	D-Arg	D-arginyl
Hypro	L-hydroxypropyl		
D-Hypro	D-hydroxypropyl		
Dopa	L-3,4-dihydroxy-phenylalanyl	L-<Glu	L-pyroglutamyl
D-Dopa	D-3,4-dihydroxy-phenylalanyl	-	
		D-<Glu	D-pyroglutamyl
Hyls	L- δ -hydroxylysyl	Sar	N-methylglycyl (sarcosyl)
D-Hyls	D- δ -methylalanyl		
Aib	L- α -methylalanyl (L-aminoisobutyryl)	α -Naphth	L- α -naphthylalanyl
		D- α -Naphth	D- α -naphthylalanyl
		β -Naphth	L- β -naphthylalanyl
Iql	L-isoquinolyl	D- β -Naphth	D- β -naphthylalanyl
D-Iql	D-isoquinolyl		



Virtually any suitable N-terminal and desamino alpha-carbon substitution can be used in the instant invention as represented by the various structural formulas set forth herein. Typical N-terminal and desamino alpha-carbon substitutions include, but are not limited to, those set forth in Table I.

TABLE I
N-Terminus and Desamino Alpha-Carbon Substitutions¹

<u>N-Terminus Substitutions</u>				
<u>X₁</u>	<u>X₂</u>	<u>X₃</u>	<u>a</u>	<u>b</u>
R ₁ -	R ₂ -	-	1	0
R ₁ -	R ₂ -	R ₃ -	1	1
$\begin{array}{c} \text{O} \\ \\ \text{R}_1-\text{Z}-\text{C}- \end{array}$	R ₂ -	-	1	0
$\begin{array}{c} \text{O} \\ \\ \text{R}_1-\text{C}- \end{array}$	R ₂ -	-	1	0
R ₁ -	HO-	-	1	0
$\begin{array}{c} \text{R}_2 \\ \\ \text{R}_1-\text{N}- \end{array}$	R ₃ -	-	1	0
$\begin{array}{c} \text{R}_2 \\ \\ \text{R}_1-\text{N}- \end{array} \begin{array}{c} \text{Z} \\ \\ -\text{C}- \end{array}$	R ₃ -	-	1	0
<u>Desamino Alpha-Carbon Substitutions</u>				
R-	-	-	0	0
RZ-	-	-	0	0

1. LEGEND: R, R₁, R₂, and R₃ are selected from a group consisting of hydrogen; straight and branched chain alkyl groups having from 1 to 6 carbon atoms; cycloalkyl groups having from 3 to 6 carbon atoms; benzyl; benzhydryl; trityl; aryl; alkoxybenzyl; alkoxybenzhydryl; alkoxytrityl; lower haloalkyl groups having from 1 to 6 carbon atoms; halobenzyl; halo-benzhydryl; halotrityl; haloaryl; and cyclohaloalkyl groups having from 3 to 6 carbon atoms. Preferably,



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R, R₁, R₂, and R₃ are selected from the group consisting of hydrogen and alkyl groups having from 1 to 6 carbon atoms. More preferably, R, R₁, R₂, and R₃ are selected from the group consisting of hydrogen and alkyl groups having 1 to 2 carbon atoms.

Z is selected from a group consisting of oxygen and sulfur. Z is preferably oxygen.

Virtually any suitable C-terminal and descaboxo alpha-carbon substitution can be used in the instant invention as represented by the various structural formulas set forth herein. Typical C-terminal and descarboxy alpha-carbon substitutions include, but are not limited to, those also set forth in Table II.

TABLE II

C-Terminus and Descarboxy
Alpha-Carbon Substitutions¹
C-Terminus Substitutions

-OR

R₁-N-R₂ -

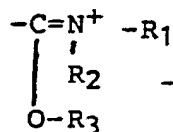
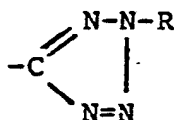
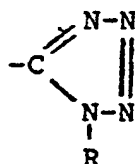
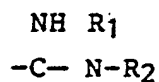
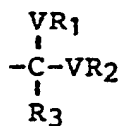
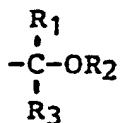
-R

R₁-C-R₂R₃R₁ R₂-N- N-R₃R₁ R₂O-N- N-C-R₃

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Descarboxy Alpha-Carbon Substitutions

-R



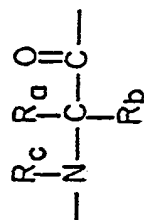
1. LEGEND: R, R₁, R₂, and R₃ are as defined in Table I, supra.

V is selected from a group consisting of oxygen, sulfur, and nitrogen. V is preferably oxygen.

The structure of amino acid residues employed in the peptides of this invention are set forth in Table III. Typical homologues and analogues of these amino acid residues which can also be employed in the peptides of this invention include, but are not limited to, those listed in Table III.



TABLE III
L or D Amino Acid Residue



NAME	NATURAL SUBSTITUENTS			SUBSTITUENTS OF HOMOLOGUES & ANALOGUES ¹		
	R_a -H	R_b -H	R_c -H	R_a -H	R_b -H	R_c -H
Gly	-CH ₃	-H	-H	-CH ₃	-H	-H
Ala	-CH ₃	-H	-H	-CH ₃	-H	-H
Val	-CH(CH ₃) ₂	-H	-H	-CH(CH ₃) ₂	-H	-H
Leu	-CH ₂ CH(CH ₃) ₂	-H	-H	-CH ₂ CH(CH ₃) ₂	-H	-H

TABLE III (contd.)

NAME	NATURAL SUBSTITUENTS			SUBSTITUENTS OF HOMOLOGUES & ANALOGUES ¹		
	$\overline{R_a}$	$\overline{R_b}$	$\overline{R_c}$	$\overline{R_a}$	$\overline{R_b}$	$\overline{R_c}$
Ile	$\begin{array}{c} \text{CH}_3 \\ \\ -\text{CH}-\text{C}_2\text{H}_5 \end{array}$	-H	-H	$\begin{array}{c} \text{CH}_3 \\ \\ -\text{CH}-\text{C}_2\text{H}_5 \end{array}$	U	U ₁
NIle	$-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$	-H	-H	$-(\text{CH}_2)_{n+1}\text{CH}_3$	U	U ₁
				$=\text{CH}(\text{CH}_2)_n\text{CH}_3$	-	U
Pro	$\begin{array}{c} \text{CH}_2 \\ \\ -\text{CH}_2-\text{CH}_2-\text{CH}_2 \\ \end{array}$	-H	$\begin{array}{c} \text{CH}_2 \\ \end{array}$	$\begin{array}{c} \text{CH}_2 \\ \\ -(\text{CH}_2)_n-\text{CH}_2 \\ \end{array}$	U	$\begin{array}{c} \text{CH}_2 \\ \end{array}$
				$\begin{array}{c} \text{CH}_2 \\ \\ -\text{CH}=\text{CH} \\ \end{array}$	U	$\begin{array}{c} \text{CH} \\ \end{array}$
				$\begin{array}{c} \text{CH}_2 \\ \\ -\text{CHOU}-\text{CH}_2 \\ \end{array}$	U ₁	$\begin{array}{c} \text{CH}_2 \\ \end{array}$
				$\begin{array}{c} (\text{CH}_2)_n \\ \\ -\text{B}-(\text{CH}_2) \\ \end{array}$	U	$\begin{array}{c} (\text{CH}_2) \\ \end{array}$
Ser	$-\text{CH}_2\text{OH}$	-H	-H	$-(\text{CH}_2)_{n+1}\text{OH}$	U ₁	U ₂
Thr	$-\text{CHOH}-\text{CH}_3$	-H	-H	$-(\text{CH}_2)_{n+1}\text{CHOH}-\text{CH}_3$	U ₁	U ₂

TABLE III (contd.)

NAME	NATURAL SUBSTITUENTS			SUBSTITUENTS OF HOMOLOGUES & ANALOGUES ¹		
	<u>R_a</u>	<u>R_b</u>	<u>R_c</u>	<u>R_a</u>	<u>R_b</u>	<u>R_c</u>
Cys	-CH ₂ SH	-H	-H	-(CH ₂) _{n+1} SU	U ₁	U ₂
Met	-CH ₂ CH ₂ SCH ₃	-H	-H	-(CH ₂) _{n+1} SO ₃ H	U	U ₁
				-(CH ₂) _{n+1} SCH ₃	U	U ₁
				=CH(CH ₂) _{n+1} SCH ₃	-	U ₁
				-(CH ₂) _{n+1} SOCH ₃	U	U ₁
				=CH(CH ₂) _{n+1} SOCH ₃	-	U ₁
				-(CH ₂) _{n+1} SO ₂ CH ₃	U	U ₁
Asp	-CH ₂ CO ₂ H	-H	-H	=CH(CH ₂) _{n+1} SO ₂ CH ₃	-	U
				-(CH ₂) _{n+1} CO ₂ U	U ₁	U ₂
				=CH(CH ₂) _n CO ₂ U	-	U ₁
Glu	-(CH ₂) ₂ CO ₂ H	-H	-H	-(CH ₂) _{n+1} CO ₂ U	U ₁	U ₂
Asn	-CH ₂ CONH ₂	-H	-H	-(CH ₂) _{n+1} CONR ₁ R ₂	U	U ₁
				=CH(CH ₂) _n CONR ₁ R ₂	-	U
Gln	-(CH ₂) ₂ CONH ₂	-H	-H	-(CH ₂) _{n+1} CONR ₁ R ₂	U	U ₁

TABLE III (contd.)

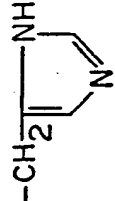
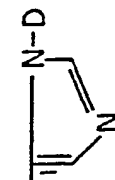
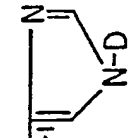
NAME	NATURAL SUBSTITUENTS			SUBSTITUENTS OF HOMOLOGUES & ANALOGUES ¹		
	<u>R_a</u>	<u>R_b</u>	<u>R_c</u>	<u>R_a</u>	<u>R_b</u>	<u>R_c</u>
Arg	$-(CH_2)_3NH-C(=NH)-NH_2$	-H	-H	$-(CH_2)_{n+1}NU-C(=ND)-N-D_1$ D_2	U_1	U_2
				$-(CH_2)_{n+1}NUC(=N-D_2)-N-D_3$ D_1	U_1	U_2
Lys	$-(CH_2)_4NH_2$	-H	-H	$-(CH_2)_{n+1}N-U_2$ U_1	U	U_3
				$-(CH_2)_{n+1}N^+-U_2$ U_1 U_3	U	U_4
His	$-CH_2$ 	-H	-H	$-(CH_2)_{n+1}$ 	U	U_1
				$-(CH_2)_{n+1}$ 	U	U_1

TABLE III (contd.)

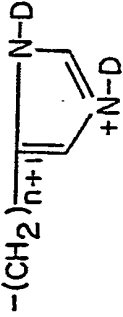
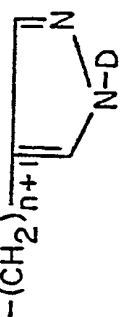
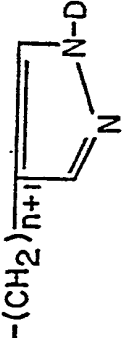
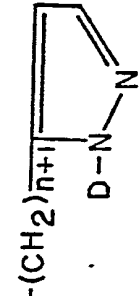
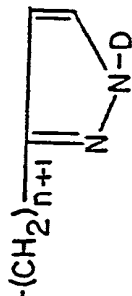
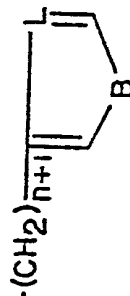
NAME	NATURAL SUBSTITUENTS			SUBSTITUENTS OF HOMOLOGUES & ANALOGUES ¹		
	<u>R_a</u>	<u>R_b</u>	<u>R_c</u>	<u>R_a</u>	<u>R_b</u>	<u>R_c</u>
					U	U ₁
					U	U ₁
					U	U ₁
					U	U ₁
					U	U ₁
					U	U ₁

TABLE III (contd.)



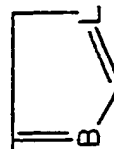
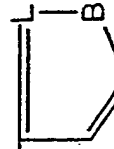
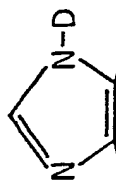
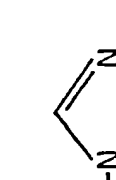
NAME	NATURAL SUBSTITUENTS			SUBSTITUENTS OF HOMOLOGUES & ANALOGUES ¹		
	$\underline{R_a}$	$\underline{R_b}$	$\underline{R_c}$	$\underline{R_a}$	$\underline{R_b}$	$\underline{R_c}$
				$-(CH_2)_{n+1}$		U_1
				$-(CH_2)_{n+1}$		U_1
				$-(CH_2)_{n+1}$		U_1
				$-(CH_2)_{n+1}$		U_1
					U	U_1
					U	U_1

TABLE III (contd.)

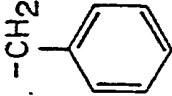
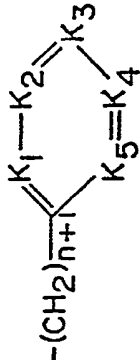
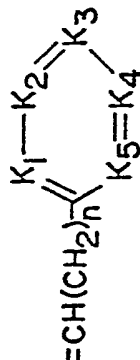
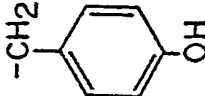
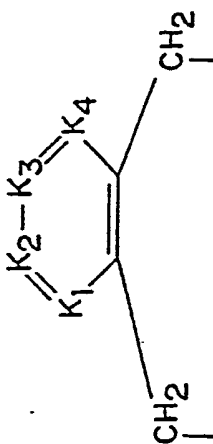
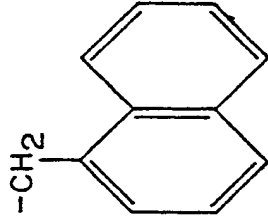
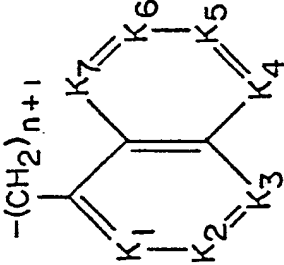
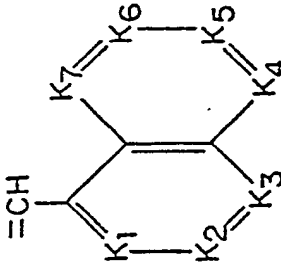
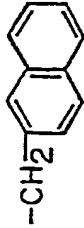
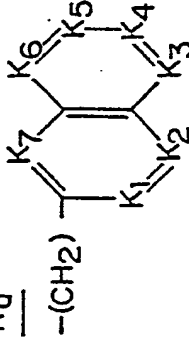
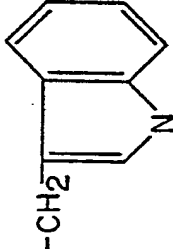
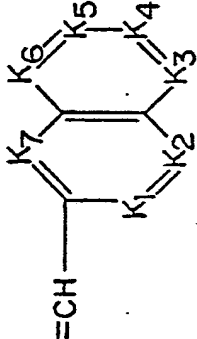
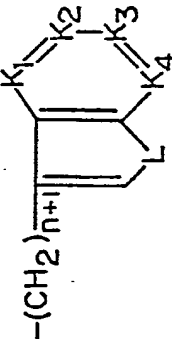
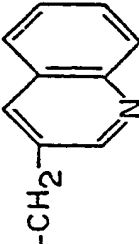
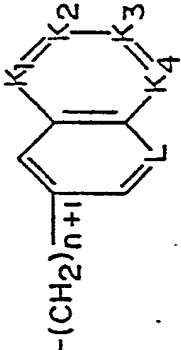
NAME	NATURAL SUBSTITUENTS			SUBSTITUENTS OF HOMOLOGUES & ANALOGUES ¹		
	<u>R_a</u>	<u>R_b</u>	<u>R_c</u>	<u>R_a</u>	<u>R_b</u>	<u>R_c</u>
Phe		-H	-H		U	U ₁
					-	U ₁
Tyr		-H	-H		See Phe above	U ₁

TABLE III (contd.)

NAME	NATURAL SUBSTITUENTS			SUBSTITUENTS OF HOMOLOGUES & ANALOGUES ¹		
	<u>R_a</u>	<u>R_b</u>	<u>R_c</u>	<u>R_a</u>	<u>R_b</u>	<u>R_c</u>
α -Naphth		-H	-H		U	U ₁
					-	U

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TABLE III (contd.)

NAME	NATURAL SUBSTITUENTS			SUBSTITUENTS OF HOMOLOGUES & ANALOGUES ¹		
	R_a	R_b	R_c	R_a	R_b	R_c
β -Naphth		-H	-H		U	U ₁
Trp		-H	-H		-	U
Sar	-H	-H	-CH ₃		U	U ₁
Iql		-H	-H		U	U ₁

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1. LEGEND: U, U₁, U₂, U₃ and U₄ are selected from a group consisting of hydrogen, alkyl groups having from 1-10 carbon atoms, and benzyl.

B, B₁, and B₂ are selected from a group consisting of -N-D, O, S.

D, D₁, D₂, and D₃ are selected from a group consisting of hydrogen, methyl, ethyl, propyl, benzyl, formyl, and tosyl.

K₁, K₂, K₃, K₄, K₅, K₆, and K₇ are N or -C-G, provided that adjacent positions are not both N.

G is selected from a group consisting of hydrogen, halogen, -OU, -OR_X, -SR_X, -R_X, -SO₃R_X, -B(OH)₂, -RN_XSOR_Y, -NR_XR_Y, -C≡N, -N(R_X)COR_Y, wherein R_X and R_Y are selected from a group consisting of hydrogen and straight and branched alkyl groups containing 1-6 carbon atoms, and substituted straight and branched alkyl groups containing 1-6 carbon atoms, wherein the substituents include, but are not limited to, one or more halo, hydroxy, amino, and mercapto groups.

L is -N or -N⁺-D.

R₁ and R₂ are as defined in Table I.

n is an integer from 0 to 4.



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The term "pharmaceutically acceptable salts", as used herein, refers to the non-toxic alkali metal, alkaline earth metal and ammonium salts commonly used in the pharmaceutical industry including, but not limited to, the sodium, potassium, lithium, calcium, magnesium, barium, ammonium and protamine salts which are prepared by methods well known in the art. The term also includes non-toxic acid addition salts which are generally prepared by reacting the compounds of this invention with a suitable organic or inorganic acid. Representative salt include, but are not limited to, the hydrochloride, hydrobromide, sulfate, bisulfate, acetate, oxalate, valerate, oleate, laurate, borate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, napsylate, and the like.

Preferably, the peptides of formula I of this invention have the amino acid sequence wherein a is 0 or 1, b is 0 and X_1 and X_2 are selected from a group consisting of $-R$, $-OR$, and $RC(O)-$, wherein R is selected from a group consisting of hydrogen and straight and branched chain alkyl groups containing 1-6 carbon atoms; A_1 and A_4 are selected from the group consisting of histidyl, tryptophyl, phenylalanyl, tyrosyl, homologues and analogues thereof, and, with respect to A_1 , the desamino forms thereof; A_2 and A_5 are selected from the group consisting of D-histidyl, D-tryptophyl, D-phenylalanyl, D-tyrosyl, homologues and analogue thereof, and, with respect to A_5 , the descarboxy forms thereof; A_3 is selected from the group consisting of glycyl, alanyl, seryl, asparaginyl, prolyl, D-alanyl, D-seryl, D-asparaginyl, D-prolyl, and homologues and analogues thereof; Y is selected from a group consisting of $-CH_2OH$, $-OR$, and $-NR_1R_2$, wherein R, R_1 , and R_2 are selected from a group consisting of hydrogen and straight and branched



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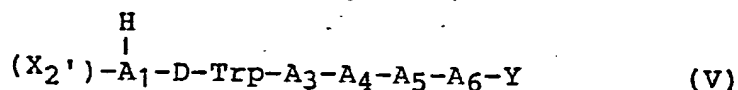
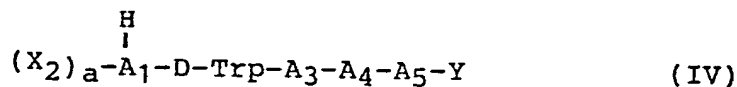
chain alkyl groups containing 1-6 carbon atoms; and the pharmaceutically acceptable salts thereof.

Preferably, the peptides of formulas II and III of this invention have the amino acid sequence wherein a is 0 or 1, b is 0 and X_1' , X_2' , X_1'' , and X_2'' are selected from a group consisting of -R, -OR, and $RC(O)-$, wherein R is selected from a group consisting of hydrogen and straight and branched chain alkyl groups containing 1-6 carbon atoms; A_1 and A_4 are selected from the group consisting of histidyl, tryptophyl, phenylalanyl, tyrosyl, homologues and analogues thereof, and, with respect to A_1 , the desamino forms thereof; A_2 and A_5 are selected from the group consisting of D-histidyl, D-tryptophyl, D-phenylalanyl, D-tyrosyl, homologues and analogues thereof; A_3 is selected from the group consisting of glycyl, alanyl, seryl, asparaginy, prolyl, D-alanyl, D-seryl, D-asparaginy, D-prolyl, and homologues and analogues thereof; A_6 is selected from the group consisting of arginine, lysine, ornithine, histidine, aspartic acid, glutamic acid, asparagine, glutamine, D-arginine, D-lysine, D-orthithine, D-histidine, D-aspartic acid, D-glutamic acid, D-asparagine, D-glutamine, D-arginine, homologues and analogues thereof, and the descarboxy forms thereof; Y is selected from a group consisting of $-CH_2OH$, -OR, and $-NR_1R_2$, wherein R, R_1 , and R_2 are selected from a group consisting of hydrogen and straight and branched chain alkyl groups containing 1-6 carbon atoms; and the pharmaceutically acceptable salts thereof.

More preferably, the peptides of (a) formula I and (b) formulas II and III of this invention have the amino acid sequence represented by formulas IV and V, respectively:



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wherein a is 0 or 1; X_2 is selected from the group consisting of R- and RC(O)- ; wherein R is selected from the group consisting of hydrogen and alkyl groups containing 1-2 carbon atoms; A_1 is selected from the group consisting of tyrosyl, O-methyltyrosyl, histidyl, 3-N-methylhistidyl, p-chlorophenylalanyl, and the desamino forms thereof; A_3 is selected from the group consisting of alanyl, seryl, and D-alanyl; A_4 is selected from the group consisting of tryptophyl and tyrosyl; A_5 is selected from the group consisting of D-phenylalanyl, D-histidyl, D-tyrosyl, and D-p-chlorophenylalanyl; A_6 is selected from the group consisting of arginine, homo-arginine, lysine, ornithine, aspartic acid, glutamic acid, asparagine, glutamine, and D-lysine; and Y is selected from the group consisting of -OR and -NHR, wherein R is selected from the group consisting of hydrogen and alkyl groups containing 1-2 carbon atoms; and the pharmaceutically acceptable salts thereof.

Peptides within the scope of the instant invention include, but are not limited to, those set forth in Table IV and the desamino and/or descarboxy forms thereof, wherein the respective positions of (a) X_1 , X_2 , X_3 , (b) X_1' , X_2' , X_3' , and (c) X_1'' , X_2'' , X_3'' are set forth in formulas I-III, respectively.

TABLE IV

(X_1 -, (X_2 -) $_a$, (X_3 -) $_b$)-His-D-Trp-Ala-Trp-D-Phe-Y
 (X_1 -, (X_2 -) $_a$, (X_3 -) $_b$)-His-D-5-Br-Trp-Ala-Trp-D-Phe-Y
 (X_1 -, (X_2 -) $_a$, (X_3 -) $_b$)-His-D-Trp-Ala-5-Br-Trp-D-Phe-Y



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(X₁-, (X₂-)_a, (X₃-)_b)-1-N-Me-His-D-Trp-Ala-Trp-D-Phe-Y
 (X₁-, (X₂-)_a, (X₃-)_b)-3-N-Me-His-D-Trp-Ala-Trp-D-Phe-Y
 (X₁-, (X₂-)_a, (X₃-)_b)-Arg-D-Trp-Ala-Iql-D-Phe-Y
 (X₁-, (X₂-)_a, (X₃-)_b)-Lys-D-Trp-Ala-Trp-D-Phe-Y
 (X₁-, (X₂-)_a, (X₃-)_b)-His-D-Trp-Ser-Trp-D-Phe-Y
 (X₁-, (X₂-)_a, (X₃-)_b)-Tyr-D-Trp-D-Ala-Trp-D-His-Y
 (X₁-, (X₂-)_a, (X₃-)_b)-Tyr-D-Trp-Ala-Trp-D-1-N-Me-His-Y
 (X₁-, (X₂-)_a, (X₃-)_b)-Tyr-D-Trp-Ala-Trp-D-3-N-Me-His-Y
 (X₁-, (X₂-)_a, (X₃-)_b)-Tyr-D-Trp-Ala-Trp-D-Arg-Y
 (X₁-, (X₂-)_a, (X₃-)_b)-Tyr-D-Trp-Ala-Trp-D-Lys-Y
 (X₁-, (X₂-)_a, (X₃-)_b)-Tyr-D-Trp-D-Ser-Trp-D-Lys-Y
 (X₁-, (X₂-)_a, (X₃-)_b)-His-D-Trp-Ala-Trp-D-His-Y
 (X₁-, (X₂-)_a, (X₃-)_b)-Arg-D-Phe-Val-Tyr-D-Lys-Y
 (X₁-, (X₂-)_a, (X₃-)_b)-Tyr-D-Tyr-Met-Phe-D-Arg-Y
 (X₁-, (X₂-)_a, (X₃-)_b)-Phe-D-Phe-Gln-Phe-D-1-N-Me-His-Y
 (X₁-, (X₂-)_a, (X₃-)_b)-His-D-Trp-Ile-Tyr-D-Trp-Y
 (X₁-, (X₂-)_a, (X₃-)_b)-α-Naphth-D-Trp-D-Ala-β-Naphth-D-Phe-Y
 (X₁-, (X₂-)_a, (X₃-)_b)-β-Naphth-D-Lys-D-His-His-D-Arg-Y
 (X₁'-, (X₂'-)_a, (X₃'-)_b)-His-D-Trp-Ala-Trp-D-Phe-Lys-Y
 (X₁'-, (X₂'-)_a, (X₃'-)_b)-His-D-5-Br-Trp-Ala-Trp-D-Phe-Lys-Y
 (X₁'-, (X₂'-)_a, (X₃'-)_b)-His-D-Trp-Ala-5-Br-Trp-D-Phe-Lys-Y
 (X₁'-, (X₂'-)_a, (X₃'-)_b)-1-N-Me-His-D-Trp-Ala-Trp-D-Phe-
 Asn-Y
 (X₁'-, (X₂'-)_a, (X₃'-)_b)-3-N-Me-His-D-Trp-Ala-Trp-D-Phe-
 Lys-Y
 (X₁'-, (X₂'-)_a, (X₃'-)_b)-Arg-D-Trp-Ala-Iql-D-Phe-Arg-Y
 (X₁'-, (X₂'-)_a, (X₃'-)_b)-Lys-D-Trp-Ala-Trp-D-Phe-Glu-Y
 (X₁'-, (X₂'-)_a, (X₃'-)_b)-His-D-Trp-Ser-Trp-D-Phe-Lys-Y
 (X₁'-, (X₂'-)_a, (X₃'-)_b)-Tyr-D-Trp-D-Ala-Trp-D-His-Gln-Y
 (X₁'-, (X₂'-)_a, (X₃'-)_b)-Tyr-D-Trp-Ala-Trp-D-1-N-Me-His-
 Met-Y
 (X₁'-, (X₂'-)_a, (X₃'-)_b)-Tyr-D-Trp-Ala-Trp-D-3-N-Me-His-
 Pro-Y
 (X₁'-, (X₂'-)_a, (X₃'-)_b)-Tyr-D-Trp-Ala-Trp-D-Arg-His-Y
 (X₁'-, (X₂'-)_a, (X₃'-)_b)-Tyr-D-Trp-Ala-Trp-D-Lys-Ser-Y
 (X₁'-, (X₂'-)_a, (X₃'-)_b)-Tyr-D-Trp-D-Ser-Trp-D-Lys-Phe-Y



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$(X_1'-, (X_2'-)_a, (X_3'-)_b)$ -His-D-Trp-Ala-Trp-D-His-Trp-Y
 $(X_1'-, (X_2'-)_a, (X_3'-)_b)$ -Arg-D-Phe-Val-Tyr-D-Lys-D-Lys-Y
 $(X_1'-, (X_2'-)_a, (X_3'-)_b)$ -Tyr-D-Tyr-Met-Phe-D-Arg-D-Orn-Y
 $(X_1'-, (X_2'-)_a, (X_3'-)_b)$ -Phe-D-Phe-Gln-Phe-D-1-N-Me-His-D-Asp-Y
 $(X_1'-, (X_2'-)_a, (X_3'-)_b)$ -His-D-Trp-Ile-Tyr-D-Trp-D-Glu-Y
 $(X_1'-, (X_2'-)_a, (X_3'-)_b)$ - α -Naphth-D-Trp-D-Ala- β -Naphth-D-Phe-Val-Y
 $(X_1'-, (X_2'-)_a, (X_3'-)_b)$ - β -Naphth-D-Lys-D-His-His-D-Arg-Thr-Y

The peptides of the instant invention can be prepared by solution methods known in the art or by using standard solid-phase techniques. The solid-phase synthesis, for example, can be commenced from the C-terminal end of the peptide using an α -amino protected amino acid. A suitable starting material can be prepared, for instance, by attaching the required α -amino acid to a chloromethyl resin, a hydroxymethyl resin, a benzhydrylamine (BHA) resin, or a p-methylbenzylhydramine (p-Me-BHA) resin. One such chloromethyl resin is sold under the tradename BIO-BEADS SX-1 by Bio Rad Laboratories, Richmond, California. The preparation of the hydroxymethyl resin is described by Bodansky et al., Chem. Ind. (London) 38, 1597 (1966). The BHA resin has been described by Pietta and Marshall, Chem. Commn. 650 (1970) and is commercially available from Beckman Instruments, Inc., Palo Alto, California in the hydrochloride form thereof (BHA·HCl).

In the solid-phase preparation of the compounds of this invention, a protected amino acid can be coupled to a resin with the aid of a coupling agent. After the initial coupling, the α -amino protecting group can be removed by a choice of reagents including trifluoroacetic acid (TFA) or hydrochloric acid (HCl) solutions in or-



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ganic solvents at room temperature. After removal of the α -amino protecting group, the remaining protected amino acids can be coupled stepwise in the desired order. Each protected amino acid can be generally reacted in about a 3-fold excess using an appropriate carboxyl group activator such as dicyclohexylcarbodiimide (DCC) in solution, for example, in methylene chloride (CH_2Cl_2)-dimethylformamide (DMF) mixtures.

After the desired amino acid sequence has been completed, the desired peptide can be cleaved from the resin support by treatment with a reagent such as hydrogen fluoride (HF) which not only cleaves the peptide from the resin, but also cleaves all remaining side-chain protecting groups. When a chloromethyl resin or hydroxymethyl resin is used, HF treatment results in the formation of the free peptide acids of formulas I-III ($\text{Y} = -\text{COOH}$). When the BHA or p-Me-BHA resin is used, HF treatment results directly in the free peptide amides of formulas I-III ($\text{Y} = -\text{CONH}_2$). Alternatively, when the chloromethylated or hydroxymethyl resin is employed, the side-chain protected peptide can be cleaved from the resin by treatment of the peptide-resin with ammonia to give the desired side-chain protected amide or with an alkylamine to give a side-chain protected alkylamide or dialkylamide. Side-chain protection can then be removed in the usual fashion by treatment with HF to give the free peptide amides, alkylamides, or dialkylamides.

In preparing the esters of this invention, the resins used to prepare the acids of formulas I-III ($\text{Y} = -\text{COOH}$) can be employed and the side-chain protected peptide can be cleaved with a base and an appropriate alcohol, i.e., methanol. Side-chain protecting groups can then be removed in the usual fashion by treatment with HF to obtain the desired ester.

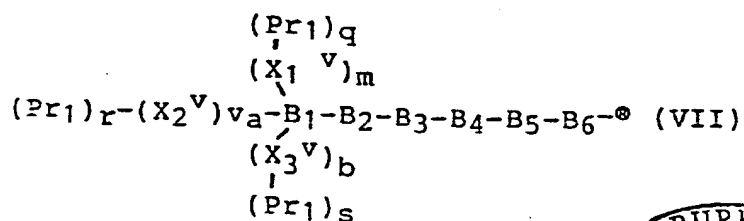
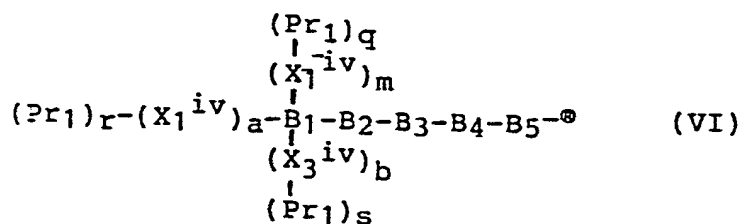


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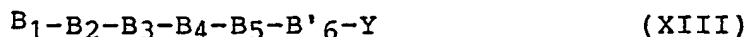
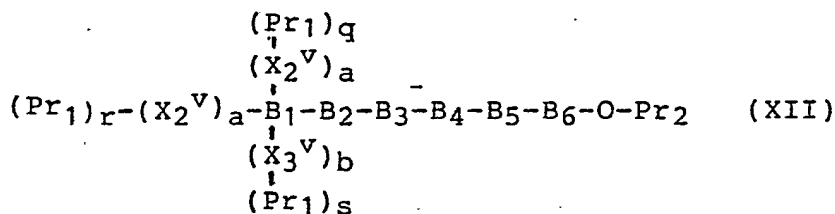
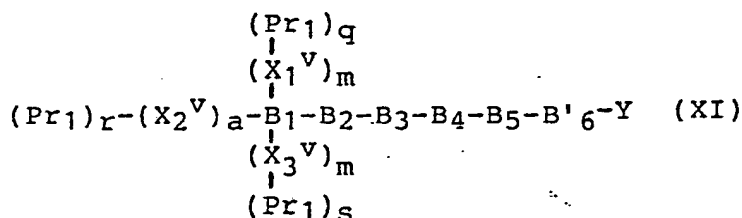
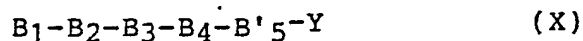
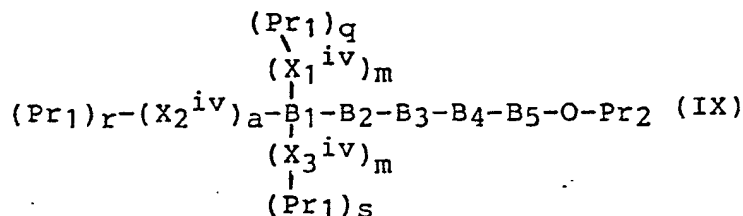
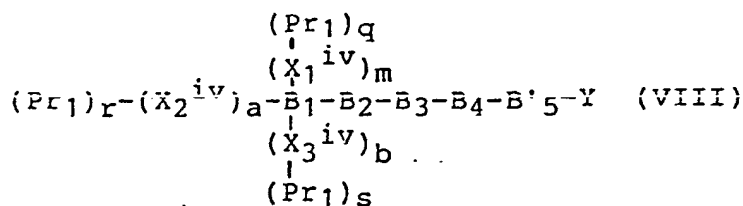
The solid-phase procedure discussed above is well known in the art and has been essentially described by Stewart and Young, Solid Phase Peptide Synthesis, Freeman and Co., San Francisco (1969).

Some of the well known solution methods which can be employed to synthesize the peptides of the instant invention are set forth in Bodansky et al., Peptide Synthesis, 2nd Edition, John Wiley & Sons, New York, N.Y. (1976).

Accordingly, also within the scope of the instant invention are intermediate compositions prepared during the synthesis of the novel peptides of formulas I-II. Intermediate compositions prepared via solid-phase techniques are the peptide-resin compounds of formulas VI-VII and intermediate compositions prepared via solution techniques are the protected peptide-compounds of formulas VIII-XIII:



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wherein Pr_1 is an α -amino protecting group; q , r , and s are each either 0 or 1; a and b are as defined above; m is either 0 or 1; X_1^{iv} , X_2^{iv} , X_3^{iv} , and X_1^{v} , X_2^{v} , and X_3^{v} are selected from a group consisting of N-terminal and desamino alpha-carbon substitutions and radicals; B_1 and B_4 are selected from a group consisting of histidyl, arginyl, lysyl, α -naphthylalanyl, β -naphthylalanyl, iso-quinolyl, tyrosyl, tryptophyl, phenylalanyl, homologues

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and analogues thereof, the side-chain protected forms thereof, and, with respect to B_1 , the desamino forms thereof; B_2 , B_5 , and B'_5 are selected from a group consisting of D-histidyl, D-arginyl, D-lysyl, D- α -naphthylalanyl, D- β -naphthylalanyl, D-isoquinolyl, D-tyrosyl, D-tryptophyl, D-phenylalanyl, homologues and analogues thereof, the side-chain protected forms thereof, and, with respect to B'_5 , the descarboxy forms thereof; B_3 is selected from a group consisting of glycyl, alanyl, valyl, leucyl, isoleucyl, prolyl, seryl, threonyl, methionyl, aspartyl, glutamyl, asparaginyl, glutaminyl, histidyl, D-alanyl, D-valyl, D-leucyl, D-isoleucyl, D-prolyl, D-seryl, D-threonyl, D-methionyl, D-aspartyl, D-glutamyl, D-asparaginyl, D-glutaminyl, D-histidyl, homologues and analogues thereof, and the side-chain protected forms thereof; B_6 and B'_6 are selected from a group consisting of amino acid residues of the L- and D- configuration, homologues and analogues thereof, the side-chain protection forms thereof; \oplus is a resin; Y is as defined above; and Pr_2 is a carboxyl protecting group; provided that (a) when (1) a is 1 and b and m are 0 and X_2^{iv} is selected from the group consisting of -H and -CH₃; (2) B_1 and B_4 are selected from the group consisting of tyrosyl, tryptophyl, phenylalanyl, and the side-chain protected forms thereof; (3) B_3 is selected from the group consisting of glycyl, alanyl, valyl, leucyl, isoleucyl, prolyl, seryl, threonyl, methionyl, aspartyl, glutamyl, asparaginyl, glutaminyl, histidyl and the side-chain protected forms thereof; and, with respect to formulas (VIII) and (X), (4) Y is selected from the group consisting of -NR₁R₂, -OR, and -CH₂OR, wherein each R, R₁, and R₂ is selected from a group consisting of hydrogen and straight and branched chain alkyl groups containing 1-6 carbon atoms; then at least one of B_2 , B_5 , and B'_5 is selected such that it is not from a group consisting of D-tyrosyl, D-tryptophyl, D-phenylalanyl,



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and, with respect to B'₅, the descarboxy forms thereof, and the side-chain protected forms thereof; (b) when (1) a is 1 and b and m are 0 and X₂^{iv} is selected from the group consisting of -H and -CH₃; (2) B₂ and B₅ or B'₅ are selected from the group consisting of D-tyrosyl, D-tryptophyl, D-phenylalanyl, and, with respect to B'₅, the descarboxy forms thereof, and the side-chain protected forms thereof; (3) B₃ is selected from the group consisting of glycyl, alanyl, valyl, leucyl, isoleucyl, prolyl, seryl, threonyl, methionyl, aspartyl, glutamyl, asparaginy, glutaminy, histidyl, and the side-chain protected forms thereof; and, with respect to formulas (VIII) and (X), (4) Y is selected from the group consisting of -NR₁R₂, -OR, and -CH₂OR, wherein each R, R₁, and R₂ is selected from a group consisting of hydrogen and straight and branched chain alkyl groups containing 1-6 carbon atoms; then at least one of B₁ and B₄ is selected such that it is not from a group consisting of tyrosyl, tryptophyl, phenylalanyl, and the side-chain protected forms thereof; (c) when (1) a is 1 and b and m are 0 and X₂^v is selected from the group consisting of -H, -CH₃, and -CHOCH₃; (2) B₁ and B₄ are selected from the group consisting of tyrosyl, tryptophyl, phenylalanyl, and the side-chain protected forms thereof; (3) B₃ is selected from the group consisting of glycyl, alanyl, valyl, leucyl, isoleucyl, seryl, threonyl, methionyl, asparaginy, glutaminy, and the side-chain protected forms thereof; (4) B₆ is selected from the group consisting of asparaginy, glutaminy, glutamyl, arginy, lysyl, seryl, threonyl, and the side-chain protected forms thereof; and, with respect to formulas (XI) and (XIII), (5) Y is selected from the group consisting of -NR₁R₂, -OR, and -CH₂OR, wherein each R, R₁, and R₂ is selected from a group consisting of hydrogen and straight and branched chain alkyl groups containing 1-6 carbon atoms; then at least one of B₂ and



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B₅ is selected such that it is not from a group consisting of D-tyrosyl, D-tryptophyl, D-phenylalanyl, and the side-chain protected forms thereof; and (d) when (1) a is 1 and b and m are 0 and X₂^v is selected from the group consisting of -H, -CH₃, and -CHOCH₃; (2) B₂ and B₅ are selected from the group consisting of D-tyrosyl, D-tryptophyl, D-phenylalanyl, and the side-chain protected forms thereof; (3) B₃ is selected from the group consisting of glycyl, alanyl, valyl, leucyl, isoleucyl, prolyl, seryl, threonyl, methionyl, aspartyl, glutamyl, asparaginyl, glutaminyl, histidyl, and the side-chain protected forms thereof; (4) B₆ is selected from the group consisting of asparaginyl, glutaminyl, glutamyl, arginyl, lysyl, seryl, threonyl, and the side-chain protected forms thereof; and, with respect to formulas (XI) and (XIII), (5) Y is selected from the group consisting of -NR₁R₂, -OR, and -CH₂OR, wherein each R, R₁, and R₂ is selected from a group consisting of hydrogen and straight and branched chain alkyl groups containing 1-6 carbon atoms; then at least one of B₁ and B₄ is selected such that it is not from a group consisting of tyrosyl, tryptophyl, phenylalanyl, and the side-chain protected forms thereof.

Preferably, the peptide resins of formula VI, and the protected peptide-compounds of formulas VIII-X, have the amino acid sequence wherein B₁ and B₄ are selected from the group consisting of histidyl, tryptophyl, phenylalanyl, tyrosyl, homologues and analogues thereof, the side-chain protected forms thereof, and, with respect to B₁, the desamino forms thereof; B₂, B₅, and B'₅ are selected from the group consisting of D-histidyl, D-tryptophyl, D-phenylalanyl, D-tyrosyl, homologues and analogues thereof, and, with respect to B'₅, the descaboxyl forms thereof, and the side-chain protected forms thereof; and B₃ is selected from the group consisting of glycyl, alanyl, seryl, asparaginyl, prolyl, D-alanyl, D-

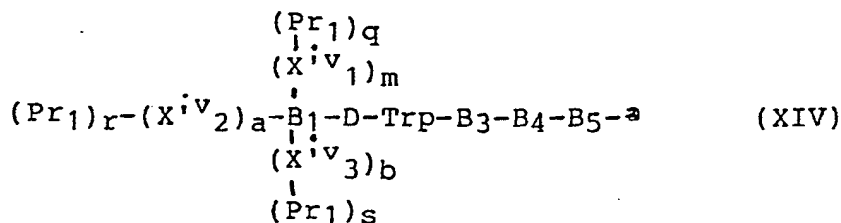


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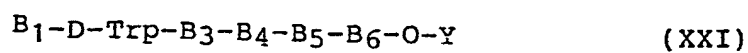
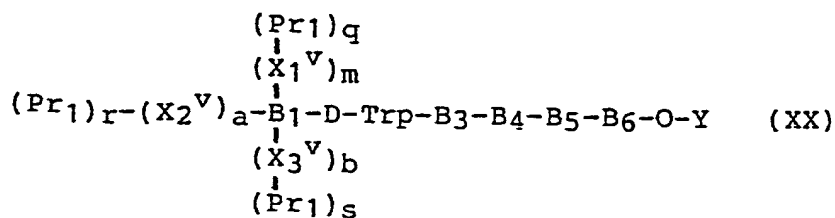
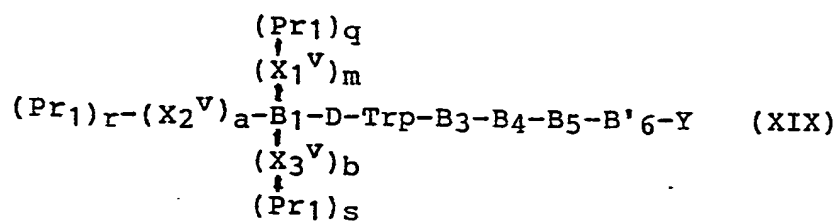
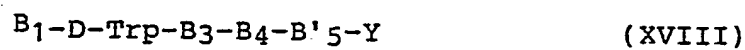
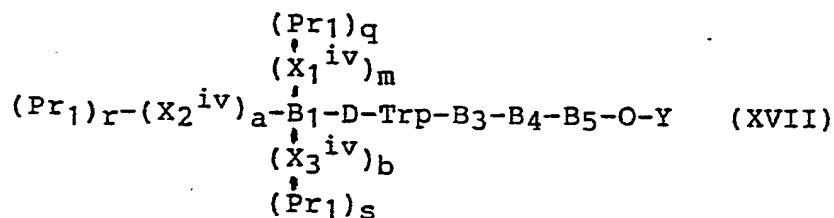
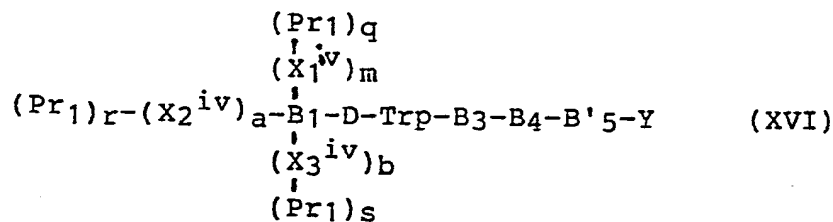
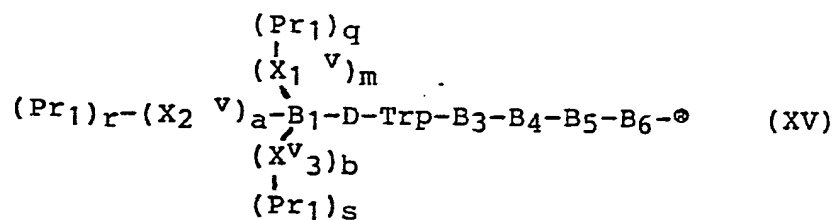
seryl, D-asparaginy, D-prolyl, homologues and analogues thereof, and the side-chain protected forms thereof.

Preferably, the peptide resins of formula VII, and the protected peptide-compounds of formulas XI-XIII, have the amino acid sequence wherein B₁ and B₄ are selected from the group consisting of histidyl, tryptophyl, phenylalanyl, tyrosyl, homologues and analogues thereof, the side-chain protected forms thereof, and, with respect to B₁, the desamino forms thereof; B₂ and B₅ are selected from the group consisting of D-histidyl, D-tryptophyl, D-phenylalanyl, D-tyrosyl, homologues and analogues thereof, and the side-chain protected forms thereof; B₃ is selected from the group consisting of glycyl, alanyl, seryl, asparaginy, prolyl, D-alanyl, D-seryl, D-asparaginy, D-prolyl, homologues and analogues thereof, and the side-chain protected forms thereof, and B₆ and B'₆ are selected from the group consisting of arginine, lysine, ornithine, histidine, aspartic acid, glutamic acid, asparagine, glutamine, D-arginine, D-lysine, D-ornithine, D-histidine, D-aspartic acid, D-glutamic acid, D-asparagine, D-glutamine, D-arginine, homologues and analogues thereof, and the descarboxy forms thereof; and, with respect to B'₆, the descarboxy forms thereof, and the side-chain protection forms thereof.

More preferably, the peptide-resins of this invention are represented by formulas XIV and XV and the protected peptide-compounds are represented by formulas XVI-XXI:



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wherein B₁ is selected from the group consisting of tyrosyl, O-methyltyrosyl, histidyl, 3-N-methylhistidyl, p-chlorophenylalanyl, the desamino forms thereof, and the side-chain protected forms thereof; B₃ is selected from the group consisting of alanyl, seryl, D-alanyl and the side-chain protected forms thereof; B₄ is selected from the group consisting of tryptophyl, tyrosyl and the side-chain protected forms thereof; B₅ and B'₅ are selected from the group consisting of D-phenylalanyl, D-histidyl, D-tyrosyl, D-p-chlorophenylalanyl, and, with respect to B'₅ the descarboxy forms thereof, and the side-chain protected forms thereof and B₆ and B'₆ are selected from the group consisting of arginine, homoarginine, lysine, ornithine, aspartic acid, glutamic acid, asparagine, glutamine, and D-lysine.

Suitable α -amino acid protecting groups Pr₁, include, but are not limited to, tertiary-butyloxycarbonyl (BOC), isoamyloxycarbonyl (AOC), o-nitrophenylsulfonyl (NPS), fluoroenylmethyloxycarbonyl (FMOC), o-nitropyridinylsulfonyl (NPYS), and biphenylpropyloxycarbonyl (BPOC).

Suitable carboxyl protecting groups, Pr₂, include, but are not limited to, salts (e.g., Li⁺, Na⁺ CS⁺, etc.), methyl, ethyl, benzyl, benzhydryl, substituted benzyl, phthalimidomethyl, tertiary butyl, phenacyl, phenyl, 4-picolyl, 2-methylthioethyl, 2(p-toluenesulfonyl)ethyl, 2(p-nitrothiophenyl)ethyl, p-methylthiophenyl, and hydrazides.

In addition to the resins, ®, noted above, other resins include, but are not limited to, phenylacetamidomethyl (PAM), chloromethyl, and poly-N-acrylpyrrolidine resins.



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Virtually any suitable N-terminal and desamino alpha-carbon substitution and radical can be used in the instant invention. Typical N-terminal and desamino alpha-carbon substitutions and radicals include, but are not limited to, those set forth in Table V.



TABLE V
N-Terminus and Desamino Substitutions and Radicals¹

X_1^v	X_2^v	X_3^v	<u>a</u>	<u>b</u>	<u>m</u>	<u>q</u>	<u>r</u>	<u>s</u>
<u>N-Terminus Substitutions and Radicals</u>								
$R_1-(R_1 \neq H)$	$R_2-(R_2 \neq H)$	—	1	0	1	0	0	0
—	R_2-	—	1	0	0	1	0	0
$R_1-(R_1 \neq H)$	$R_2-(R_2 \neq H)$	$R_3-(R_3 \neq H)$	1	1	1	0	0	0
R_1-	R_2-	R_3-	1	1	1	0	0	0
$R_1-Z-\overset{O}{\parallel}C-$	R_2-	—	1	0	1	0	0	0
$R_1-\overset{O}{\parallel}C-$	R_2-	—	1	0	1	0	0	0
R_1-	—O—	—	1	0	1	0	1	0
$R_1-N-(R_1 \& R_2 \neq H)$	$R_3-(R_3 \neq H)$	—	1	0	1	0	0	0
$R_1-N-(R_1 \& R_2 \neq H)$	—	—	0	0	1	0	1	0

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TABLE V (Contd.)

$\underline{X_1'}$	$\underline{X_2'}$	$\underline{X_3'}$	\underline{a}	\underline{b}	\underline{m}	\underline{q}	\underline{r}	\underline{s}
$\begin{array}{c} R_2 \\ \\ -N- \end{array}$	—	—	0	0	1	1	1	0
$\begin{array}{c} R_2 \\ \\ -N- \end{array}$	$R_3-(R_3 \neq H)$	—	1	0	1	1	0	0
$\begin{array}{c} R_2 \\ \\ -N- \end{array}$	$R_3-(R_3 \neq H)$	—	1	0	1	0	0	0
$\begin{array}{c} R_2 \quad Z \\ \\ R_1-N-C-(R_1 \& R_2 \neq H) \end{array}$	—	—	0	0	1	0	1	0
$\begin{array}{c} R_2 \quad Z \\ \\ R_1-N-C-(R_1 \& R_2 \neq H) \end{array}$	—	—	0	0	1	0	1	0
$\begin{array}{c} R_2 \quad Z \\ \\ -N-C- \end{array}$	$R_3-(R_3 \neq H)$	—	1	0	1	1	0	0
<u>Desamino Substituents and Radicals</u>								
R—	—	—	0	0	1	0	0	0
RZ—(R≠H)	—	—	0	0	1	0	0	0
—Z—	—	—	0	0	1	1	0	0

1. LEGEND: R, R₁, R₂, R₃, and Z are as defined in TABLE I, supra.

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The growth hormone releasing peptides of formulas I and II and combinations thereof (including combinations of formula III) with at least one growth promoting agent are useful in vitro as unique tools for understanding how growth hormone secretion is regulated at the pituitary level. This includes use in the evaluation of many factors thought or known to influence growth hormone secretion such as age, sex, nutritional factors, glucose, amino acids, fatty acids, as well as fasting and non-fasting states. In addition, the peptides of this invention can be used in the evaluation of how other hormones modify growth hormone releasing activity. For example, it has already been established that somatostatin inhibits growth hormone release. Other hormones that are important and in need of study as to their effect on growth hormone release include the gonadal hormones, e.g., testosterone, estradiol, and progesterone; the adrenal hormones, e.g., cortisol and other corticoids, epinephrine and norepinephrine; the pancreatic and gastrointestinal hormones, e.g., insulin, glucagon, gastrin, secretin; the vasoactive intestinal peptides, e.g., bombesin; and the thyroid hormones, e.g., thyroxine and triiodothyronine. These peptides and combinations of this invention can also be employed to investigate the possible negative or positive feedback effects of some of the pituitary hormones, e.g., growth hormone and endorphin peptides, on the pituitary to modify growth hormone release. Of particular scientific importance is the use of these peptides to elucidate the subcellular mechanisms mediating the release of growth hormone.

The peptides and combinations of this invention can also be administered to animals, including man, to release growth hormone in vivo. For example, the peptides can be administered to commercially important



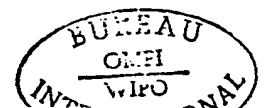
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animals such as swine, cattle, sheep and the like to accelerate and increase their rate and extent of growth, and to increase milk production in such animals. In addition, these peptides can be administered to humans in vivo as a diagnostic tool to directly determine whether the pituitary is capable of releasing growth hormone. For example, the peptides and combinations can be administered in vivo to children. Serum samples taken before and after such administration can be assayed for growth hormone. Comparison of the amounts of growth hormone in each of these samples would be a means for directly determining the ability of the patient's pituitary to release growth hormone.

Accordingly, the present invention includes within its scope pharmaceutical compositions comprising, as an active ingredient, at least one of the peptides or combinations of this invention in association with a pharmaceutical carrier or diluent. Optionally, the active ingredient of the pharmaceutical compositions can comprise a growth promoting agent in addition to at least one of the peptides of formulas I or II or another composition which exhibits a different activity, e.g., an antibiotic or other pharmaceutically active material.

Growth promoting agents include, but are not limited to, TRH, diethylstilbesterol, theophylline, enkephalins, E series prostaglandins, compounds disclosed in U.S. Patent 3,239,345, e.g., zeranol, and compounds disclosed in U.S. Patent 4,036,979, e.g., sulbenox.

The peptides of this invention can be administered by oral, parenteral (e.g., intramuscular, intraperitoneal, intravenous or subcutaneous injection, or implant), nasal, vaginal, rectal, sublingual, or topical



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routes of administration and can be formulated in dosage forms appropriate for each route of administration.

Solid dosage forms for oral administration include capsules, tablets, pills, powders and granules. In such solid dosage forms, the active compound is admixed with at least one inert pharmaceutically acceptable carrier such as sucrose, lactose, or starch. Such dosage forms can also comprise, as is normal practice, additional substances other than inert diluents, e.g., lubricating agents such as magnesium stearate. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering agents. Tablets and pills can additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, the elixirs containing inert diluents commonly used in the art, such as water. Besides such inert diluents, compositions can also include adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring, and perfuming agents.

Preparations according to this invention for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, or emulsions. Examples of non-aqueous solvents or vehicles are propylene glycol, polyethylene glycol, vegetable oils, such as olive oil and corn oil, gelatin, and injectable organic esters such as ethyl oleate. Such dosage forms may also contain adjuvants such as preserving, wetting, emulsifying, and dispersing agents. They may be sterilized by, for example, by filtration through a bacteria-retaining filter, by incorporating sterilizing agents into the compositions, by irradiating the compositions, or by heating the compositions. They can also be manufactured in the form

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of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use.

Compositions for rectal or vaginal administration are preferably suppositories which may contain, in addition to the active substance, excipients such as cocoa butter or a suppository wax.

Compositions for nasal or sublingual administration are also prepared with standard excipients well known in the art.

The dosage of each active ingredient in the compositions of this invention may be varied; however, it is necessary that the amount of the active ingredient be such that a suitable dosage form is obtained. The selected dosage depends upon the desired therapeutic effect, on the route of administration, and on the duration of the treatment. Generally, dosage levels per active ingredient of between 0.001 to 10 mg/kg. of body weight daily are administered to animals, e.g., mammals, to obtain effective release of growth hormone.

The following examples are provided for the purpose of further illustration only and are not intended to be limitations on the disclosed invention.

Example 1

Synthesis of H₂-His-D-Trp-Ala-Trp-D-Phe-NH₂

Para-methylbenzhydrylamine hydrochloride (p-Me-BHA.HCl) resin was placed in a reaction vessel. The following procedure, starting at step 6, was then employed in conjunction with a Beckman brand Peptide Synthesizer Model No. 990 in preparing the peptide H₂-His-D-Trp-Ala-Trp-D-Phe-NH₂. The synthesis was started



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at step 6 because there was no amino acid present in the resin and one need only neutralize the resin which was initially in the HCl form.

1. Wash with methylene chloride (CH_2Cl_2) for 1.5 minutes, three times.
2. Treat with trifluoroacetic acid - methylene chloride (40% TFA/ CH_2Cl_2 , V/V) containing 0.1% indole for 1.5 minutes.
3. Repeat Step 2 for 20 minutes.
4. Wash with chloroform (CHCl_3) for 1.5 minutes, three times.
5. Wash with 30% ethanol-methylene chloride (30% EtOH/ CH_2Cl_2 , V/V) for 1.5 minutes, two times.
6. Wash with CH_2Cl_2 for 1.5 minutes, three times.
7. Treat with 10% triethylamine in CH_2Cl_2 (10% TEA/ CH_2Cl_2 , V/V) for 1.5 minutes.
8. Repeat Step 7 for 10 minutes.
9. Wash with CH_2Cl_2 for 1.5 minutes, three times.
10. Add to the washed resin 2.5 equivalents of the appropriate protected amino acid in dimethyl formamide-methylene chloride (DMF- CH_2Cl_2).
11. Add 0.5N dicyclohexylcarbodiimide in CH_2Cl_2 (DCC/ CH_2Cl_2); more than 2.5 equivalents.
12. Rinse addition funnel with CH_2Cl_2 and add rinse to the reaction vessel.
13. Stir the reagents in Steps 10-12 for 2 hours or more.
14. Wash with CH_2Cl_2 for 1.5 minutes, three times.
15. Wash with DMF for 1.5 minutes.
16. Wash with CH_2Cl_2 for 1.5 minutes, two times.
17. Test by ninhydrin reaction according to the procedure of Kaiser et al., Annal. Biochem., 34:595 (1970).
18. If Step 17 shows complete reaction, repeat the above procedures starting from Step 1 employing the next



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protected amino acid. If Step 17 shows incomplete reaction, repeat Steps 7-17.

The above procedure was employed using the following sequence of amino acids:

Boc-D-Phe

Boc-Trp

Boc-Ala

Boc-D-Trp

Boc-His(Tos*)

*Tos denotes p-toluenesulfonyl.

After completion of the synthesis of the desired peptide resin, the reaction vessel containing the peptide resin was then placed in a dessicator and dried overnight under a vacuum. The dried peptide resin was removed from the reaction vessel and placed in another vessel suitable for HF cleavage. This latter vessel also contained a magnetic stirring bar. A quantity of anisole sufficient to wet the peptide resin was added to this vessel. The vessel was next connected to an HF line and placed under a vacuum to remove any air therein. The vessel was then cooled to about -78°C . with a dry ice-acetone bath. Doubly distilled HF (about 10 ml/gm of peptide resin) was added to the vessel. The dry ice-acetone bath was then removed from the vessel and replaced by an ice-water bath. The vessel's contents were vigorously stirred for about 45 minutes while the vessel remained immersed in the ice-water bath. Most of the HF in the vessel was then removed by water aspiration. After the majority of HF was removed by water aspiration, the remaining HF and anisole were removed via a vacuum pump.



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The vessel's contents were washed with about 100 ml of ether to further remove any residual anisole.

The peptide was removed from the resin by extraction with aqueous acetic acid (aq·HOAc). The aq·HOAc was lyophilized off to yield a fluffy peptide powder.

The peptide was then purified by partition chromatography or counter current distribution (CCD) employing a butanol:HOAc:water (4:1:5) system. When further purification was necessary, a Pharmacia LH-20 brand chromatography column was also employed.

Example 2

Synthesis of H₂-Tyr-D-Trp-Ala-Trp-D-His-NH₂

The procedure set forth in Example 1 can be employed to synthesize the peptide H₂-Tyr-D-Trp-Ala-Trp-D-His-NH₂ employing the following sequence of amino acids:

Boc-D-His(Tos)

Boc-Trp

Boc-Ala

Boc-D-Trp

Boc-Tyr(BrZ*)

*BrZ denotes o-bromobenzyloxycarbonyl

Example 3

Synthesis of H₂-His-D-Trp-Ala-Trp-D-Tyr-NH₂

The procedure set forth in Example 1 can be employed to synthesize the peptide H₂-His-D-Trp-Ala-Trp-D-Tyr-NH₂ employing the following sequence of amino acids:

Boc-D-Tyr (BrZ)

Boc-Trp



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Boc-Ala

Boc-D-Trp

Boc-His (Tos)

Example 4Synthesis of H₂-His-D-Trp-Ala-Trp-D-His-NH₂

The procedure set forth in Example 1 can be employed to synthesize the peptide H₂-His-D-Trp-Ala-Trp-D-His-NH₂ employing the following sequence of amino acids:

Boc-D-His (Tos)

Boc-Trp

Boc-Ala

Boc-D-Trp

Boc-His (Tos)

Example 5Synthesis of H₂-Tyr-D-Trp-Ala-Trp-D-p-Cl-Phe-NH₂

The procedure set forth in Example 1 can be employed to synthesize the peptide H₂-Tyr-D-Trp-Ala-Trp-D-p-Cl-Phe-NH₂ employing the following sequence of amino acids:

Boc-D-p-Cl-Phe

Boc-Trp

Boc-Ala

Boc-D-Trp

Boc-Tyr(BrZ)

Example 6Synthesis of H₂-Tyr-D-Trp-D-Ala-Trp-D-Phe-NH₂

The procedure set forth in Example 1 can be employed to synthesize the peptide H₂-Tyr-D-Trp-D-Ala-Trp-D-Phe-NH₂ employing the following sequence of amino acids:



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Boc-D-Phe
Boc-Trp
Boc-D-Ala
Boc-D-Trp
Boc-Tyr(BrZ)

Example 7Synthesis of H₂-p-Cl-Phe-D-Trp-Ala-Trp-D-Phe-NH₂

The procedure set forth in Example 1 can be employed to synthesize the peptide H₂-p-Cl-Phe-D-Trp-Ala-Trp-D-Phe-NH₂ employing the following sequence of amino acids:

Boc-D-Phe
Boc-Trp
Boc-Ala
Boc-D-Trp
Boc-p-Cl-Phe

Example 8Synthesis of H-desaminoTyr-D-Trp-Ala-Trp-D-Phe-NH₂

The procedure set forth in Example 1 can be employed to synthesize the peptide H-desaminoTyr-D-Trp-Ala-Trp-D-Phe-NH₂ employing the following sequence of amino acids:

Boc-D-Phe
Boc-Trp
Boc-Ala
Boc-D-Trp
3(p-OH-phenyl)propanoic acid



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Example 9

H
Synthesis of $\text{CH}_3\text{CO-Tyr-D-Trp-Ala-Trp-D-Phe-NH}_2$

The procedure set forth in Example 1 can be employed with several modifications to synthesize the peptide

H
 $\text{CH}_3\text{CO-Tyr-D-Trp-Ala-Trp-D-Phe-NH}_2$ employing the following sequence of amino acids:

Boc-D-Phe
Boc-Trp
Boc-Ala
Boc-D-Trp
Boc-Tyr(BrZ)

The modifications consisted of the following additional steps after the last protected amino acid, Boc-Tyr(BrZ) was added to the peptide resin:

19. The Boc group was removed from the peptide resin by TFA.

20. The resulting peptide resin was washed with CH_2Cl_2 for 1.5 minutes, two times.

21. Acetic anhydride (2.5 molar excess) and 2.5 molar excess of pyridine were added and stirred for about 10 minutes.

22. Repeat Step 20.

The same drying and purification steps as used in Example 1 were then employed to obtain the desired peptide.

Example 10

Synthesis of $\text{H}_2\text{-O-Me-Tyr-D-Trp-Ala-Trp-D-Phe-NH}_2$

The procedure set forth in Example 1 can be



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employed to synthesize the peptide H_2 -O-Me-Tyr-D-Trp-Ala-Trp-D-Phe-NH₂ employing the following sequence of amino acids:

Boc-D-Phe
Boc-Trp
Boc-Ala
Boc-D-Trp
Boc-O-Me-Tyr(BrZ)

Example 11

Synthesis of H_2 -Tyr-D-Trp-Ala-Trp-D-Phe-Met-NH₂

BHA·HCl resin was placed in a reaction vessel. The following procedure was then employed in conjunction with a Beckman brand Peptide Synthesizer Model No. 990 in preparing the hexapeptide H_2 -Tyr-D-Trp-Ala-Trp-D-Phe-Met-NH₂:

1. Methylene chloride (CH₂Cl₂; about 10 ml/gm of BHA·HCl resin) was added to the reaction vessel. The BHA·HCl resin was washed with vigorous stirring for about 1.5 minutes. The CH₂Cl₂ solution was then drained from the reaction vessel. This washing step was repeated once.

2. A triethylamine solution ((Et₃N)/CH₂Cl₂ (10:90); about 10 ml/gm BHA·HCl resin) was added to the washed BHA·HCl resin in the reaction vessel. The resulting mixture was vigorously stirred for about 1.5 minutes. The solution was then drained from the reaction vessel.

3. Another Et₃N/CH₂Cl₂ (10:90) solution (about 10 ml/gm BHA·HCl) was added to the reaction vessel. The BHA·HCl resin was neutralized by vigorous stirring for about 20 minutes. The solution was then drained from the reaction vessel.

4. CH₂Cl₂ (about 10 ml/gm of BHA·HCl resin) was added to the reaction vessel. The resulting mixture was



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vigorously stirred for about 1.5 minutes. The solution was then drained from the reaction vessel. This procedure was repeated an additional two times.

5. Tertiarybutyloxycarbonyl-methionine (Boc-Met; about 2.5 times the theoretical amount of the total binding capacity of the BHA·HCl resin originally placed in the reaction vessel) in about 50 ml of dimethylformamide-methylene chloride solution (DMF-CH₂Cl₂ (1:9)) was added to the reaction vessel. The resulting mixture was vigorously stirred for about 1.5 minutes.

6. A 0.5 molar (M) dicyclohexylcarbodiimide (DCC) in CH₂Cl₂ solution (about 2.5 times the theoretical amount of total binding capacity of the BHA·HCl resin originally placed in the reaction vessel) was added to the reaction vessel. The resulting mixture was vigorously stirred until a negative ninhydrin test was obtained (about 120 minutes). The solution was then drained from the reaction vessel.

7. CH₂Cl₂ (about 10 ml/gm of BHA·HCl resin) was added to the reaction vessel. The resulting solution was vigorously stirred for about 1.5 minutes. The solution was then drained from the reaction vessel. This washing procedure was repeated once.

8. DMF (about 10 ml/gm of BHA·HCl resin) was added to the reaction vessel. The resulting mixture was stirred for about 1.5 minutes. The solution was then drained from the reaction vessel.

9. CH₂Cl₂ (about 10 ml/gm of BHA·HCl resin) was added to the reaction vessel. The resulting mixture was vigorously stirred for about 1.5 minutes. The solution was then drained from the reaction vessel. This washing procedure was repeated an additional two times.

10. A trifluoroacetic acid/methylene chloride solution (TFA/CH₂Cl₂ (40:60); about 10 ml/gm of BHA·HCl resin) was added to the reaction vessel. The resulting mixture was vigorously stirred for about 1.5 minutes.



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The solution was then drained from the reaction vessel.

11. Another TFA/CH₂Cl₂ (40:60) solution (about 10 ml/gm of BHA·HCl resin) was added to the reaction vessel. The resulting mixture was vigorously stirred for about 20 minutes. The solution was then drained from the reaction vessel.

12. CH₂Cl₂ (about 10 ml/gm of BHA·HCl resin) was added to the reaction vessel. The resulting solution was vigorously stirred for about 1.5 minutes. The solution was then drained from the reaction vessel. This washing procedure was repeated once.

13. A triethylamine solution ((Et₃N)/CH₂Cl₂ (10:90); about 10 ml/gm BHA HCl resin) was added to the washed BHA·HCl resin in the reaction vessel. The resulting mixture was vigorously stirred for about 1.5 minutes. The solution was then drained from the reaction vessel.

14. Another Et₃N/CH₂Cl₂ (10:90) solution (about 10 ml/gm BHA·HCl) was added to the reaction vessel. The BHA·HCl resin was neutralized by vigorous stirring for about 20 minutes. The solution was then drained from the reaction vessel.

15. Chloroform (CHCl₃; about 10 ml/gm of BHA·HCl resin) was added to the reaction vessel. The resulting mixture was vigorously stirred for about 1.5 minutes. The solution was then drained from the reaction vessel.

16. An ethanol/methylene chloride solution (EtOH/CH₂Cl₂ (30:70); about 10 ml/gm of BHA·HCl resin) was added to the reaction vessel. The resulting mixture was vigorously stirred for about 1.5 minutes. The solution was then drained from the reaction vessel. This washing step was repeated once.

Steps 4 through 16 were then repeated employing the following sequence of amino acids:

BUREAU
OMPI

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Boc-D-Phe

Boc-Trp

Boc-Ala

Boc-D-Trp.

Boc-Tyr (BrZ*)

*BrZ denotes o-bromobenzyloxycarbonyl

After completion of the synthesis of the desired peptide resin, the reaction vessel containing the peptide resin was then placed in a dessicator and dried overnight under a vacuum. The dried peptide resin was removed from the reaction vessel and placed in another vessel suitable for HF cleavage. This latter vessel also contained a magnetic stirring bar. A quantity of anisole sufficient to wet the peptide resin was added to this vessel. The vessel was next connected to an HF and placed under a vacuum to remove any air therein. The vessel was then cooled to about -78°C . with a dry ice-acetone bath. Doubly distilled HF (about 10 ml/gm of peptide resin) was added to the vessel. The dry ice-acetone bath was then removed from the vessel and replaced by an ice-water bath. The vessel's contents were vigorously stirred for about 45 minutes while the vessel remained immersed in the ice-water bath. Most of the HF in the vessel was then removed by water aspiration, the remaining HF and anisole were removed via a vacuum pump.

The vessel's contents were washed with about 100 ml of ether to further remove any residual anisole.

The peptide was removed from the resin by extraction with 30% aqueous acetic acid (aq.HOAc). The aq.HOAc was lyophilized off to yield a fluffy peptide powder.



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The peptide was then purified by partition chromatography or counter current distribution (CCD) employing a butanol: HOAc: water (4:1:5) system. When further purification was necessary, a Pharmacia LH-20 brand chromatography column was also employed.

Example 12

Synthesis of H₂-Tyr-D-Trp-Ala-Trp-D-Phe-Thr-NH₂

The procedure set forth in Example 11 was employed to synthesize the hexapeptide H₂-Tyr-D-Trp-Ala-Trp-D-Phe-Thr-NH₂ employing the following sequence of amino acids:

Boc-Thr(Bzl*)

Boc-D-Phe

Boc-Trp

Boc-Ala

Boc-D-Trp

Boc-Tyr(BrZ)

*Bzl denotes benzyl.

Example 13

Synthesis of H₂-Tyr-D-Trp-Ala-Trp-D-Phe-Gln-OH

The procedure set forth in Example 11 was employed to synthesize the hexapeptide H₂-Tyr-D-Trp-Ala-Trp-D-Phe-Gln-OH employing the following sequence of amino acids:

Boc-Glu- α -benzyl ester

Boc-D-Phe

Boc-Trp

Boc-Ala

Boc-D-Trp

Boc-Tyr(BrZ)



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Example 14Synthesis of H₂-Tyr-D-Trp-Ala-Trp-D-Phe-Gln-NH₂

The procedure set forth in Example 11 was employed with several modifications to synthesize the hexapeptide H₂-Tyr-D-Trp-Ala-Trp-D-Phe-Gln-NH₂. The modifications consisted of:

(1) Omitting steps 2-4 of Example 11 and replacing them with a single step which entailed dissolving Boc-Gln (about 5 times the theoretical amount of the total binding of the BHA·HCl resin originally placed in the reaction vessel) in 10 ml of CH₃Cl₂-DMF solution (3:2) present in a round bottom flask. The flask and its contents was cooled in ice and then DCC (about 1.25 times the theoretical amount of the total binding capacity of the BHA·HCl resin originally placed in the reaction vessel) was added to the flask. The resulting mixture was stirred in an ice bath for about 25-30 minutes. The DCC urea precipitate formed by the reaction between Boc-Gln and DCC was separated from the supernatant via filtration. The supernature comprising the CH₂Cl₂-DMF solution having a symmetrical anhydride (also formed by the reaction between Boc-Gln and DCC) dissolved therein was added to the reaction vessel. The resulting mixture was stirred until a negative ninhydrin test was obtained. The solution was then drained from the reaction vessel.

(2) After completing steps 7 through 16 of Example 11, steps 4 through 16 of Example 11 were then repeated, without modification, employing the following sequence of amino acids:

Boc-D-Phe
Boc-Trp
Boc-Ala
Boc-D-Trp
Boc-Tyr(BrZ)



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Example 15Synthesis of H₂-Tyr-D-Trp-Ala-Trp-D-Phe-Asn-NH₂

The procedure set forth in Example 11 was employed with several modifications to synthesize the hexapeptide H₂-Tyr-D-Trp-Ala-Trp-D-Phe-Asn-NH₂. The modifications consisted of:

(1) Omitting steps 2-4 of Example 11 and replacing them with a simple step which entailed dissolving Boc-Asn (about 5 times the theoretical amount of the total binding capacity of the BHA·HCl resin originally placed in the reaction vessel) in about 50 ml of DMF in a suitable flask. The resulting solution was added to the reaction vessel to form a mixture. The mixture was vigorously stirred until a negative ninhydrin test was obtained. The solution was then drained from the reaction vessel.

(2) After completing steps 7 through 16 of Example 11, steps 4 through 16 of Example 11 were then repeated, without modification, employing the following sequence of amino acids:

Boc-D-Phe
Boc-Trp
Boc-Ala
Boc-Tyr(BrZ)

Example 16Synthesis of H₂-His-D-Trp-D-Phe-Lys-NH₂

Para-methylbenzhydrylamine hydrochloride (p-Me-BHA·HCl) resin was placed in a reaction vessel. The following procedure, starting at step 6, was then employed in conjunction with a Beckman brand Peptide Synthesizer Model 990 in preparing the peptide H₂-His-D-Trp-Ala-Trp-D-Phe-Lys-NH₂. The synthesis was started at step 6 because there was no amino acid present in the



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resin and one need only neutralize the resin which was initially in the HCl form.

1. Wash with methylene chloride (CH_2Cl_2) for 1.5 minutes, three times.
2. Treat with trifluoroacetic acid - methylene chloride (40% TFA/ CH_2Cl_2 , V/V) containing 0.1% indole for 1.5 minutes.
3. Repeat Step 2 for 20 minutes.
4. Wash with chloroform (CHCl_3) for 1.5 minutes, three times.
5. Wash with 30% ethanol-methylene chloride (30% EtOH/ CH_2Cl_2 , V/V) for 1.5 minutes, two times.
6. Wash with CH_2Cl_2 for 1.5 minutes, three times.
7. Treat with 10% triethylamine in CH_2Cl_2 (10% TEA/ CH_2Cl_2 , V/V) for 1.5 minutes.
8. Repeat Step 7 for 10 minutes.
9. Wash with CH_2Cl_2 for 1.5 minutes, three times.
10. Add to the washed resin 2.5 equivalents of the appropriate protected amino acid in dimethyl formamide-methylene chloride (DMF- CH_2Cl_2).
11. Add 0.5N dicyclohexylcarbodiimide in CH_2Cl_2 (DCC/ CH_2Cl_2); more than 2.5 equivalents.
12. Rinse addition funnel with CH_2Cl_2 and add rinse to the reaction vessel.
13. Stir the reagents in Steps 10-12 for 2 hours or more.
14. Wash with CH_2Cl_2 for 1.5 minutes, three times.
15. Wash with DMF for 1.5 minutes.
16. Wash with CH_2Cl_2 for 1.5 minutes, two times.
17. Test by ninhydrin reaction according to the procedure of Kaiser et al., Annal. Biochem., 34:595 (1970).
18. If Step 17 shows complete reaction, repeat the above procedures starting from Step 1 employing the next

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protected amino acid. If Step 17 shows incomplete reaction, repeat Steps 7-17.

The above procedure was employed using the following sequence of amino acids:

Boc-Lys(ClZ⁺)

Boc-D-Phe

Boc-Trp

Boc-Ala

Boc-D-Trp

Boc-His(Tos*)

+ClZ denotes o-chloro-benzyloxycarbonyl.

*Tos denotes p-toluenesulfonyl.

After completion of the synthesis of the desired peptide resin, the reaction vessel containing the peptide resin was then placed in a dessicator and dried overnight under a vacuum. The dried peptide resin was removed from the reaction vessel and placed in another vessel suitable for HF cleavage. This latter vessel also contained a magnetic stirring bar. A quantity of anisole sufficient to wet the peptide resin was added to this vessel. The vessel was next connected to an HF line and placed under a vacuum to remove any air therein. The vessel was then cooled to about -78°C. with a dry ice-acetone bath. Doubly distilled HF (about 10 ml/gm of peptide resin) was added to the vessel. The dry ice-acetone bath was then removed from the vessel and replaced by an ice-water bath. The vessel's contents were vigorously stirred for about 45 minutes while the vessel remained immersed in the ice-water bath. Most of the HF in the vessel was then removed by water aspiration. After the majority of HF was removed by water aspiration, the remaining HF and anisole were removed via a vacuum pump.



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The vessel's contents were washed with about 100 ml of ether to further remove any residual anisole.

The peptide was removed from the resin by extraction with aqueous acetic acid (aq.HOAc). The aq.HOAc was lyophilized off to yield a fluffy peptide powder.

The peptide was then purified by partition chromatography or counter current distribution (CCD) employing a butanol:HOAc:water (4:1:5) system. When further purification was necessary, a Pharmacia LH-20 brand chromatography column was also employed.

Example 17

Synthesis of H₂-Tyr-D-Trp-Ala-Trp-D-Phe-Lys-NH₂

The procedure set forth in Example 16 can be employed to synthesize the peptide H₂-Tyr-D-Trp-Ala-Trp-D-Phe-Lys-NH₂ employing the following sequence of amino acids:

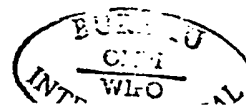
Boc-Lys(ClZ)
Boc-D-⁻ Phe
Boc-Trp
Boc-Ala
Boc-D-Trp
Boc-Tyr(BrZ*)

*BrZ denotes o-bromobenzyloxycarbonyl

Example 18

Synthesis of H₂-Tyr-D-Trp-Ala-Trp-D-Phe-Glu-NH₂

The procedure set forth in Example 16 can be employed to synthesize the peptide H₂-Tyr-D-Trp-Ala-Trp-D-Phe-Glu-NH₂ employing the following sequence of amino acids:



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Boc-Glu- γ -Bzl*

Boc-D-Phe

Boc-Trp

Boc-Ala

Boc-D-Trp

Boc-Tyr(BrZ)

* γ -Bzl denotes γ -benzyl ester

Example 19Synthesis of H₂-Tyr-D-Trp-Ala-Trp-D-Phe-Phe-NH₂

The procedure set forth in Example 16 can be employed to synthesize the peptide H₂-Tyr-D-Trp-Ala-Trp-D-Phe-Phe-NH₂ employing the following sequence of amino acids:

Boc-Phe

Boc-D-Phe

Boc-Trp

Boc-Ala

Boc-D-Trp

Boc-Tyr(BrZ)

Example 20Synthesis of H₂-Tyr-D-Trp-Gly-Trp-D-Phe-Gln-NH₂

The procedure set forth in Example 16 can be employed with one modification to synthesize the peptide H₂-Tyr-D-Trp-Gly-Trp-D-Phe-Gln-NH₂ employing the following sequence of amino acids:

Boc-Gln-ONP*

Boc-D-Phe

Boc-Trp

Boc-Gly

Boc-D-Trp

Boc-Tyr(BrZ)

*ONP denote p-nitrophenyl ester.

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The sole modification was made just in the procedure for coupling Boc-Gln-ONP to the resin. This modification consisted of the omission of step 11 of Example 16 in this one coupling procedure.

Example 21

Synthesis of H₂-His-D-Trp-Ala-Trp-D-Phe-Lys-OH

The procedure set forth in Example 16 can be employed with several modifications to synthesize the peptide H₂-His-D-Trp-Ala-Trp-D-Phe-Lys-OH employing the following sequence of amino acids:

Boc-Lys(ClZ)

Boc-D-Phe

Boc-Trp

Boc-Ala

Boc-D-Trp

Boc-His(Tos)

The modifications were as follows:

1. A hydroxymethyl resin⁻ was employed instead of the p-ME-BHA·HCl resin.
2. Steps 6-18 were modified as follows just in the procedure for coupling Boc-Lys(ClZ) to the hydroxymethyl resin:
 - a. Step 6 was the same;
 - b. Steps 7 through 9 were omitted;
 - c. Steps 10 and 11 were the same;
 - d. Step 12 was omitted and the following procedure was substituted therefor:

Add 2.5 equivalents of N,N-dimethylaminopyridine (DMAP).
 - e. Steps 13 through 16 were the same;



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f. Steps 17 and 18 were omitted and the following procedure was substituted therefor:

Dry the amino acid-resin under vacuum until a constant weight is obtained. If the final constant weight is equal to the sum of the weights of the resin and Boc-amino acid added to the reaction vessel, then add 10 equivalents of benzoyl chloride and 10 equivalents of pyridine to deactivate any unused hydroxymethyl resin. Wash as set forth in steps 15 and 16. Then employ steps 1-18 of Example 16 for remaining protected amino acids.

If the final constant weight is not equal to the sum of the weights of the resin and Boc-amino acid added to the reaction vessel, then repeat steps 10 to end as modified above in this example.

Example 22

Synthesis of H₂-His-D-Trp-Ala-Trp-D-Phe-Arg-NH₂

The procedure set forth in Example 16 can be employed to synthesize the peptide H₂-His-D-Trp-Ala-Trp-D-Phe-Arg-NH₂ employing the following sequence of amino acids:

Aoc*-Arg(Tos)

Boc-D-Phe

Boc-Trp

Boc-Ala

Boc-D-Trp

Boc-His(Tos)

*Aoc denotes isoamyloxycarbonyl

Example 23

Synthesis of H₂-His-D-Trp-Ala-Trp-D-Phe-Gln-NH₂

The procedure set forth in Example 16 can be employed with one modification to synthesize the peptide



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H₂-His-D-Trp-Ala-Trp-D-Phe-Gln-NH₂ employing the following sequence of amino acids:

Boc-Gln-ONP
Boc-D-Phe
Boc-Trp
Boc-Ala
Boc-D-Trp
Boc-His(Tos)

The sole modification was made first in the procedure for coupling Boc-Gln-ONP to the resin. This modification consisted of the omission of step 11 of Example 16 in this one coupling procedure.

Example 24

Synthesis of H₂-His-D-Trp-Ala-Trp-D-Phe-Glu-NH₂

The procedure set forth in Example 16 can be employed to synthesize the peptide H₂-His-D-Trp-Ala-Trp-D-Phe-Glu-NH₂ employing the following sequence of amino acids:

Boc-Glu-γ-Bzl
Boc-D-Phe
Boc-Trp
Boc-Ala
Boc-D-Trp
Boc-His(Tos)

Example 25

Synthesis of H₂-His-D-Trp-Ala-Trp-D-Phe-HomoArg-NH₂

The procedure set forth in Example 16 can be employed to synthesize the peptide H₂-His-D-Trp-Ala-Trp-D-Phe-HomoArg-NH₂ employing the following sequence of amino acids:



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Boc-HomoArg(Tos)

Boc-D-Phe

Boc-Trp

Boc-Ala

Boc-D-Trp

Boc-His(Tos)

Example 26Synthesis of H₂-3-N-Me-His-D-Trp-Ala-Trp-D-Phe-Lys-NH₂

The procedure set forth in Example 16 can be employed to synthesize the peptide H₂-3-N-Me-His-D-Trp-Ala-Trp-D-Phe-Lys-NH₂ employing the following sequence of amino acids:

Boc-Lys(ClZ)

Boc-D-Phe

Boc-Trp

Boc-Ala

Boc-D-Trp

Boc-3-N-Me-His

Example 27Synthesis of H₂-His-D-Trp-Ala-Trp-D-Phe-Lys-NHCH₂CH₃

The procedure set forth in Example 16 can be employed with several modifications to synthesize the peptide H₂-His-D-Trp-D-Ala-Trp-D-Phe-Lys-NHCH₂CH₃ employing the following sequence of amino acids:

Boc-Lys(ClZ)

Boc-D-Phe

Boc-Trp

Boc-D-Ala

Boc-D-Trp

Boc-His(Tos)



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The following ethylamine ($\text{CH}_3\text{CH}_2\text{NH}_2$) cleavage procedure was employed in place of the HF cleavage procedure of Example 16:

After completion of the synthesis of the desired peptide resin, the reaction vessel containing the peptide resin was then placed in a dessicator and dried overnight under a vacuum. The dried peptide resin was removed from the reaction vessel and placed in another vessel suitable for ethylamine cleavage. This latter vessel also contained a magnetic stirring bar. The vessel was placed in an ice bath and gaseous ethylamine was condensed into the vessel. The contents of the vessel was stirred overnight.

This cleavage procedure was then followed by the extraction and purifications procedures as set forth in Example 16.

Example 28

Synthesis of $\text{H}_2\text{-His-D-Trp-Ala-Trp-D-Phe-Orn-NH}_2$

The procedure set forth in Example 16 can be employed to synthesize the peptide $\text{H}_2\text{-His-D-Trp-Ala-Trp-D-Phe-Orn-NH}_2$ employing the following sequence of amino acids:

Boc-Orn(Z*)
Boc-D-Phe
Boc-Trp
Boc-Ala
Boc-D-Trp
Boc-His(Tos)

*Z denotes benzyloxycarbonyl



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Example 29Synthesis of H₂-His-D-Trp-Val-Trp-D-Phe-Lys-NH₂

The procedure set forth in Example 16 can be employed to synthesize the peptide H₂-His-D-Trp-Val-Trp-D-Phe-Lys-NH₂ employing the following sequence of amino acids:

Boc-Lys(ClZ)
Boc-D-Phe
Boc-Trp
Boc-Val
Boc-D-Trp
Boc-His(Tos)

Example 30Synthesis of H₂-His-D-Trp-Ser-Trp-D-Phe-Lys-NH₂

The procedure set forth in Example 16 can be employed to synthesize the peptide H₂-His-D-Trp-Ser-Trp-D-Phe-NH₂ employing the following sequence of amino acids:

Boc-Lys(ClZ)
Boc-D-Phe
Boc-Trp
Boc-Ser(Bzl)
Boc-D-Trp
Boc-His(Tos)

Example 31In Vitro Growth Hormone Release Study

Female rats of the CD-1 strain were housed in a constant temperature room at 24°C. with 14 hours light and 10 hours darkness. The rats were fed Purina brand rat chow ab libitum. All studies were started between 0800 and 1000 hours.



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Pituitaries were removed from 20 day old female rats. In each polytetrafluoroethylene beaker (10 ml) was incubated two pituitaries at 36°C. in 1 ml of lactated Ringer's solution in a Dubnoff Shaker (90 cycles/min.). Three beakers were employed for each dosage shown in Table VI. All medium in each beaker was removed each hour (e.g., P₁, P₂, I₃, I₄, I₅) and then fresh medium was added back to each beaker. Each medium removed was assayed for GH, in duplicate, by a standard radioimmunoassay (RIA).

The growth hormone agonist of Example 1 was not added to the incubation mediums employed during the first hour of the incubation period (P₁) or to the incubation mediums employed during the second hour of the incubation period (P₂). The growth hormone agonist of Example 1 was dissolved in dimethylsulfoxide (DMSO; 10:1, agonist:DMSO), added to each incubation medium employed during the third hour of the incubation period (I₃), to each medium employed during the third hour of the incubation period (I₃), to each medium employed during the fourth hour of the incubation period (I₄) and, when performed, to each medium employed during the fifth hour of the incubation period (I₅). The release of growth hormone was recorded as ΔGH values obtained from the three beakers per dosage level measured at I₃, I₄, and, when performed, I₅ are set forth in Table VI.

Example 32

In Vitro Growth Hormone Release Study

The procedure set forth in Example 31 was employed in an in vitro growth hormone release study of the peptide of Example 2 and the results therefrom are set forth in Table VII.



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Example 33In Vitro Growth Hormone Release Study

The procedure set forth in Example 31 was employed in an in vitro growth hormone release study of the peptide of Example 3 and the results therefrom are set forth in Table VIII.

Example 34In Vitro Growth Release Study

The procedure set forth in Example 31 was employed in an in vitro growth hormone release study of the peptide of Example 4 and the results therefrom are set forth in Table IX.

Example 35In Vitro Growth Hormone Release Study

The procedure set forth in Example 31 was employed in an in vitro growth hormone release study of the peptide of Example 5 and the results therefrom are set forth in Table X.

Example 36In Vitro Growth Hormone Release Study

The procedure set forth in Example 31 was employed in an in vitro growth hormone release study of the peptide of Example 6 and the results therefrom are set forth in Table XI.

Example 37In Vitro Growth Hormone Release Study

The procedure set forth in Example 31 was employed in an in vitro growth hormone release study of the peptide of Example 7 and the results therefrom are set forth in Table XII.



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Example 38In Vitro Growth Hormone Release Study

The procedure set forth in Example 31 was employed in an in vitro growth hormone release study of the peptide of Example 8 and the results therefrom are set forth in Table XIII.

Example 39In Vitro Growth Hormone Release Study

The procedure set forth in Example 31 was employed in an in vitro growth release study of the peptide of Example 9 and the results therefrom are set forth in Table XIV.

Example 40In Vitro Growth Hormone Release Study

The procedure set forth in Example 31 was employed in an in vitro growth hormone release study of the peptide of Example 10 and the results therefrom are set forth in Table XV.

Example 41In Vitro Growth Hormone Release Study

The procedure set forth in Example 31 was employed in an in vitro growth hormone release study of the peptide of Example 11 and the results therefrom are set forth in Table XVI.

Example 42In Vitro Growth Hormone Release Study

The procedure set forth in Example 31 was employed in an in vitro growth hormone release study of the peptide of Example 12 and the results therefrom are set forth in Table XVII.



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Example 43In Vitro Growth Hormone Release Study

The procedure set forth in Example 31 was employed in an in vitro growth hormone release study of the peptide of Example 13 and the results therefrom are set forth in Table XVIII.

Example 44In Vitro Growth Release Study

The procedure set forth in Example 31 was employed in an in vitro growth hormone release study of the peptide of Example 14 and the results therefrom are set forth in Table XIX.

Example 45In Vitro Growth Hormone Release Study

The procedure set forth in Example 31 was employed in an in vitro growth hormone release study of the peptide of Example 15 and the results therefrom are set forth in Table XX.

Example 46In Vitro Growth Hormone Release Study

The procedure set forth in Example 31 was employed in an in vitro growth hormone release study of the peptide of Example 16 and the results therefrom are set forth in Table XXI.

Example 47In Vitro Growth Hormone Release Study

The procedure set forth in Example 31 was employed in an in vitro growth hormone release study of the peptide of Example 17 and the results therefrom are set forth in Table XXII.



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Example 48In Vitro Growth Hormone Release Study

The procedure set forth in Example 31 was employed in an in vitro growth hormone release study of the peptide of Example 18 and the results therefrom are set forth in Table XXIII.

Example 49In Vitro Growth Hormone Release Study

The procedure set forth in Example 31 was employed in an in vitro growth hormone release study of the peptide of Example 19 and the results therefrom are set forth in Table XXIV.

Example 50In Vitro Growth Hormone Release Study

The procedure set forth in Example 31 was employed in an in vitro growth hormone release study of the peptide of Example 20 and the results therefrom are set forth in Table XXV.

Example 51In Vitro Growth Hormone Release Study

The procedure set forth in Example 31 was employed in an in vitro growth hormone release study of the peptide of Example 21 and the results therefrom are set forth in Table XXVI.

Example 52In Vitro Growth Hormone Release Study

The procedure set forth in Example 31 was employed in an in vitro growth hormone release study of the peptide of Example 22 and the results therefrom are set forth in Table XXVII.



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Example 53In Vitro Growth Hormone Release Study

The procedure set forth in Example 31 was employed in an in vitro growth hormone release study of the peptide of Example 23 and the results therefrom are set forth in Table XXVIII.

Example 54In Vitro Growth Hormone Release Study

The procedure set forth in Example 31 was employed in an in vitro growth hormone release study of the peptide of Example 24 and the results therefrom are set forth in Table XXIX.

Example 55In Vitro Growth Hormone Release Study

The procedure set forth in Example 31 was employed in an in vitro growth hormone release study of the peptide of Example 25 and the results therefrom are set forth in Table XXX.

Example 56In Vitro Growth Hormone Release Study

The procedure set forth in Example 31 was employed in an in vitro growth hormone release study of the peptide of Example 26 and the results therefrom are set forth in Table XXXI.

Example 57In Vitro Growth Hormone Release Study

The procedure set forth in Example 31 was employed in an in vitro growth hormone release study of the peptide of Example 27 and the results therefrom are set forth in Table XXXII.



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Example 58In Vitro Growth Hormone Release Study

The procedure set forth in Example 31 was employed in an in vitro growth hormone release study of the peptide of Example 28 and the results therefrom are set forth in Table XXXIII.

Example 59In Vitro Growth Hormone Release Study

The procedure set forth in Example 31 was employed in an in vitro growth hormone release study of the peptide of Example 29 and the results therefrom are set forth in Table XXXIV.

Example 60In Vitro Growth Hormone Release Study

The procedure set forth in Example 31 was employed in an in vitro growth hormone release study of the peptide of Example 30 and the results therefrom are set forth in Table XXXV.



TABLE VI
IN VITRO GROWTH HORMONE RELEASE

<u>Dosage²</u>	<u>ΔGH¹</u>	<u>p Value⁴</u>
<u>H₂-His-D-Trp-Ala-Trp-D-Phe-NH₂</u>		
---	-167 + 114	---
1	206 + 168	NS ³
3	288 + 79	<0.01
10	2,005 + 203	<0.001
30	3,046 + 93	<0.001

1. The mean of 9 assays given in terms of ng/ml incubation medium + standard error of the mean (SEM)
2. Given in terms of ng/ml incubation medium
3. NS denotes not significant
4. Comparison of the GH levels in medium containing growth hormone agonist analog to the GH levels in medium without the agonist



TABLE VII
IN VITRO GROWTH HORMONE RELEASE

<u>Dosage²</u>	<u>AGH¹</u>	<u>p Value⁴</u>
<u>H₂-Tyr-D-Trp-Ala-Trp-D-His-NH₂</u>		
---	-167 + 114	---
1	-179 + 323	NS ³
3	-192 + 120	NS
10	-802 + 302	NS
30	611 + 103	<0.001

1. The mean of 9 assays given in terms of ng/ml incubation medium + standard error of the mean (SEM)
2. Given in terms of ng/ml incubation medium
3. NS denotes not significant
4. Comparison of the GH levels in medium containing growth hormone agonist analog to the GH levels in medium without the agonist



TABLE VIII
IN VITRO GROWTH HORMONE RELEASE

<u>Dosage²</u>	<u>ΔGH¹</u>	<u>p Value⁴</u>
<u>H₂-His-D-Trp-Ala-Trp-D-Tyr-NH₂</u>		
---	-236 + 125	---
1	-126 + 253	NS ³
10	-99 + 230	NS
100	-238 + 133	NS
1,000	2,598 + 284	<0.001

1. The mean of 9 assays given in terms of ng/ml incubation medium + standard error of the mean (SEM)
2. Given in terms of ng/ml incubation medium
3. NS denotes not significant
4. Comparison of the GH levels in medium containing growth hormone agonist analog to the GH levels in medium without the agonist



TABLE IX
IN VITRO GROWTH HORMONE RELEASE

<u>Dosage²</u> <u>H₂-His-D-Trp-Ala-Trp-D-His-NH₂</u>	<u>ΔGH¹</u>	<u>p Value⁴</u>
---	-236 + 125	---
1	-166 + 277	NS ³
10	-369 + 152	NS
100	43 + 185	NS
1,000	1,501 + 222	<0.001

1. The mean of 9 assays given in terms of ng/ml incubation medium + standard error of the mean (SEM),
2. Given in terms of ng/ml incubation medium
3. NS denotes not significant
4. Comparison of the GH levels in medium containing growth hormone agonist analog to the GH levels in medium without the agonist



TABLE X
IN VITRO GROWTH HORMONE RELEASE

Dosage ²	ΔGH^1	p Value ⁴
H ₂ -Tyr-D-Trp-Ala-Trp-D-p-Cl-Phe-NH ₂		
---	28 + 54	---
10	-87 + 81	NS ³
30	124 + 123	NS
300	103 + 77	NS
3,000	531 + 42	<0.001
30,000	489 + 138	<0.01

1. The mean of 9 assays given in terms of ng/ml incubation medium + standard error of the mean (SEM)
2. Given in terms of ng/ml incubation medium
3. NS denotes not significant
4. Comparison of the GH levels in medium containing growth hormone agonist analog to the GH levels in medium without the agonist



TABLE XI
IN VITRO GROWTH HORMONE RELEASE

<u>Dosage²</u>	<u>AGH¹</u>	<u>p Value⁴</u>
<u>H₂-Tyr-D-Trp-D-Ala-Trp-D-Phe-NH₂</u>		
---	-257 ± 97	---
300	648 ± 210	<0.02
3,000	435 ± 143	<0.02
30,000	1,136 ± 190	<0.001

1. The mean of 6 assays given in terms of ng/ml incubation medium ± standard error of the mean (SEM)
2. Given in terms of ng/ml, incubation medium
3. Comparison of the GH levels in medium containing growth hormone agonist analog to the GH levels in medium without the agonist



TABLE XII
IN VITRO GROWTH HORMONE RELEASE

Dosage ²	ΔGH^1	p Value ⁴
<u>H₂-p-Cl-Phe-D-Trp-Ala-Trp-D-Phe-NH₂</u>		
---	-218 + 161	---
30	-260 + 271	NS ³
300	367 + 159	<0.05
1,000	714 + 110	<0.01
10,000	1,326 + 143	<0.001

1. The mean of 6 assays given in terms of ng/ml incubation medium \pm standard error of the mean (SEM) ;
2. Given in terms of ng/ml incubation medium
3. NS denotes not significant
4. Comparison of the GH levels in medium containing growth hormone agonist analog to the GH levels in medium without the agonist



TABLE XIII
IN VITRO GROWTH HORMONE RELEASE

<u>Dosage²</u>	<u>AGH¹</u>	<u>p Value³</u>
<u>H₂-desaminotyrosyl-D-Trp-Ala-Trp-D-Phe-NH₂</u>		
---	28 + 54	---
30	231 + 65	<0.02
300	432 + 109	<0.01
3,000	700 + 201	<0.01
20,000	-861 + 13	<0.001

1. The mean of 9 assays given in terms of ng/ml incubation medium + standard error of the mean (SEM)
2. Given in terms of ng/ml incubation medium
3. Comparison of the GH levels in medium containing growth hormone agonist analog to the GH levels in medium without the agonist



TABLE XIV
IN VITRO GROWTH HORMONE RELEASE

<u>Dosage²</u>	<u>ΔGH¹</u>	<u>p value⁴</u>
<u>C₁₃CO-Tyr-D-Trp-Ala-Trp-D-Phe-NH₂</u>		
---	-175 ± 58	---
10	-297 ± 79	NS ³
30	-118 ± 97	NS
300	5 ± 29	<0.02
3,000	1,594 ± 385	<0.001
30,000	1,607 ± 250	<0.001

1. The mean of 9 assays given in terms of ng/ml incubation medium + standard error of the mean (SEM)
2. Given in terms of ng/ml incubation medium
3. NS denotes not significant
4. Comparison of the GH levels in medium containing growth hormone agonist analog to the GH levels in medium without the agonist



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TABLE XV
IN VITRO GROWTH HORMONE RELEASE

Dosage ²	Δ GH ¹	p Value ⁴
H ₂ -O-Me-Tyr-D-Trp-Ala-Trp-D-Phe-NH ₂		
---	-9 + 66	---
300	177 + 166	NS ³
3,000	1,138 + 266	<0.001
30,000	892 + 388	<0.05

1. The mean of 9 assays given in terms of ng/ml incubation medium + standard error of the mean (SEM)
2. Given in terms of ng/ml, incubation medium
3. NS denotes not significant
4. Comparison of the GH levels in medium containing growth hormone agonist analog to the GH levels in medium without the agonist

TABLE XVI
IN VITRO GROWTH HORMONE RELEASE

<u>Dosage²</u>	<u>ΔGH¹</u>	<u>p Value⁴</u>
<u>H₂-Tyr-D-Trp-Ala-Trp-D-Phe-Met-NH₂</u>		
—	-148 ± 137	---
30	456 ± 151	NS ³
300	1,260 ± 245	<0.01
1,000	1,832 ± 441	<0.01

1. The mean of 6 assays given in terms of ng/ml incubation medium + standard error of the mean (SEM)
2. Given in terms of ng/ml incubation medium
3. NS denotes not significant
4. Comparison of the GH levels in medium containing growth hormone agonist analog to the GH levels in medium without the agonist



TABLE XVII
IN VITRO GROWTH HORMONE RELEASE

<u>Dosage²</u>	<u>ΔGH¹</u>	<u>p Value⁴</u>
<u>H₂-Tyr-D-Trp-Ala-Trp-D-Phe-Thr-NH₂</u>		
---	-40 + 35	---
10	94 + 69	NS ³
100	650 + 320	NS
1,000	2,167 + 591	<0.01
10,000	2,957 + 834	<0.01

1. The mean of 6 assays given in terms of ng/ml incubation medium + standard error of the mean (SEM)
2. Given in terms of ng/ml incubation medium
3. NS denotes not significant
4. Comparison of the GH levels in medium containing growth hormone agonist analog to the GH levels in medium without the agonist

TABLE XVIII
IN VITRO GROWTH HORMONE RELEASE

Dosage ²	Δ GH ¹	p Value ⁴
<u>H₂-Tyr-D-Trp-Ala-Trp-D-Phe-Gln-OH</u>		
---	-40 + 35	---
100	753 + 559	NS ³
1,000	2,759 + 515	<0.001
10,000	3,994 + 1,214	<0.01

1. The mean of 6 assays given in terms of ng/ml incubation medium \pm standard error of the mean (SEM)
2. Given in terms of ng/ml incubation medium
3. NS denotes not significant
4. Comparison of the GH levels in medium containing growth hormone agonist analog to the GH levels in medium without the agonist

TABLE XIX
IN VITRO GROWTH HORMONE RELEASE

<u>H₂-Tyr-D-Trp-Ala-Trp-D-Phe-Gln-NH₂</u>	<u>Dosage²</u>	<u>ΔGH¹</u>	<u>p Value⁴</u>
---	---	-40 + 35	---
10	10	240 + 96	NS ³
100	100	2,000 + 523	<0.01
1,000	1,000	4,235 + 785	<0.001
10,000	10,000	4,141 + 576	<0.001

1. The mean of 6 assays given in terms of ng/ml incubation medium + standard error of the mean (SEM)
2. Given in terms of ng/ml incubation medium
3. NS denotes not significant
4. Comparison of the GH levels in medium containing growth hormone agonist analog to the GH levels in medium without the agonist

TABLE XX
IN VITRO GROWTH HORMONE RELEASE

Dosage ²	ΔGH^1	p Value ³
H ₂ -Tyr-D-Trp-Ala-Trp-D-Phe-Asn-NH ₂		
---	-89 + 51	---
30	244 + 58	<0.01
100	528 + 184	<0.01
1,000	1,670 + 126	<0.001

1. The mean of 6 assays given in terms of ng/ml incubation medium + standard error of the mean (SEM)
2. Given in terms of ng/ml incubation medium
3. Comparison of the GH levels in medium containing growth hormone agonist analog to the GH levels in medium without the agonist



TABLE XXI
IN VITRO GROWTH HORMONE RELEASE

<u>Dosage²</u>	<u>AGH¹</u>	<u>p Value³</u>
<u>H₂-His-D-Trp-Ala-Trp-D-Phe-Lys-NH₂</u>		
---	167 ± 114	---
1	955 ± 272	<0.01
3	1,603 ± 305	<0.001
10	2,244 ± 173	<0.001
30	2,198 ± 358	<0.001

1. The mean of 9 assays given in terms of ng/ml incubation medium + standard error of the mean (SEM)
2. Given in terms of ng/ml incubation medium
3. Comparison of the GH levels in medium containing growth hormone agonist analog to the GH levels in medium without the agonist



TABLE XXII
IN VITRO GROWTH HORMONE RELEASE

Dosage ²	ΔGH^1	p Value ³
<u>H₂-Tyr-D-Trp-Ala-Trp-D-Phe-Lys-NH₂</u>		
---	-29 + 59	---
30	1,346 + 328	<0.001
300	2,160 + 166	<0.001
3,000	1,630 + 135	<0.001
30,000	1,318 + 247	<0.001

1. The mean of 9 assays given in terms of ng/ml incubation medium + standard error of the mean (SEM)
2. Given in terms of ng/ml incubation medium
3. Comparison of the GH levels in medium containing growth hormone agonist analog to the GH levels in medium without the agonist



TABLE XXIII
IN VITRO GROWTH HORMONE RELEASE

<u>Dosage²</u>	<u>AGH¹</u>	<u>p Value³</u>
<u>H₂-Tyr-D-Trp-Ala-Trp-D-Phe-Glu-NH₂</u>		
---	-29 + 59	---
30	224 + 98	0.05
300	472 + 104	0.05
3,000	1,404 + 179	<0.001
30,000	1,909 + 270	<0.001

1. The mean of 9 assays given in terms of ng/ml incubation medium + standard error of the mean (SEM)
2. Given in terms of ng/ml incubation medium
3. Comparison of the GH levels in medium containing growth hormone agonist analog to the GH levels in medium without the agonist



TABLE XXIV
IN VITRO GROWTH HORMONE RELEASE

Dosage ²	ΔGH^1	p Value ⁴
H ₂ -Tyr-D-Trp-Ala-Trp-D-Phe-Phe-NH ₂		
---	29 + 164	---
30	10 + 57	NS ³
300	330 + 43	NS
3,000	710 + 67	<0.01
30,000	1,484 + 135	<0.001

1. The mean of 9 assays given in terms of ng/ml incubation medium + standard error of the mean (SEM)
2. Given in terms of ng/ml incubation medium
3. NS denotes not significant
4. Comparison of the GH levels in medium containing growth hormone agonist analog to the GH levels in medium without the agonist



TABLE XXV

IN VITRO GROWTH HORMONE RELEASE

<u>Dosage²</u>	<u>ΔGH¹</u>	<u>p-Value⁴</u>
<u>H₂-Tyr-D-Trp-Ala-Trp-D-Phe-Gln-NH₂</u>		
---	-89 + 50	---
30	103 + 248	NS ³
100	167 + 65	<0.02
1,000	1,092 + 42	<0.001

1. The mean of 6 assays given in terms of ng/ml incubation medium + standard error of the mean (SEM)
2. Given in terms of ng/ml incubation medium
3. NS denotes not significant
4. Comparison of the GH levels in medium containing growth hormone agonist analog to the GH levels in medium without the agonist



TABLE XXVI
IN VITRO GROWTH HORMONE RELEASE

<u>Dosage²</u>	<u>ΔGH¹</u>	<u>p Value³</u>
<u>H₂-His-D-Trp-Ala-Trp-D-Phe-Lys-OH</u>		
---	-146 ± 67	---
1	467 ± 178	<0.01
10	330 ± 167	<0.02
100	2,441 ± 353	<0.001

1. The mean of 9 assays given in terms of ng/ml incubation medium + standard error of the mean (SEM)
2. Given in terms of ng/ml incubation medium
3. Comparison of the GH levels in medium containing growth hormone agonist analog to the GH levels in medium without the agonist



TABLE XXVII
IN VITRO GROWTH HORMONE RELEASE

<u>Dosage²</u>	<u>ΔGH¹</u>	<u>p Value³</u>
<u>H₂-His-D-Trp-Ala-Trp-D-Phe-Arg-NH₂</u>		
---	-88 + 56	---
1	805 + 152	<0.001
3	997 + 249	<0.001
10	2,064 + 346	<0.001

1. The mean of 9 assays given in terms of ng/ml incubation medium + standard error of the mean (SEM)
2. Given in terms of ng/ml incubation medium
3. Comparison of the GH levels in medium containing growth hormone agonist analog to the GH levels in medium without the agonist



TABLE XVIII
IN VITRO GROWTH HORMONE RELEASE

<u>Dosage²</u>	<u>AGH¹</u>	<u>p Value⁴</u>
<u>H₂-His-D-Trp-Ala-Trp-D-Phe-Gln-NH₂</u>		
---	-288 ± 182	---
1	5 ± 136	NS ³
3	140 ± 168	NS
10	275 ± 66	0.02

1. The mean of 6 assays given in terms of ng/ml incubation medium ± standard error of the mean (SEM)
2. Given in terms of ng/ml incubation medium
3. NS denotes not significant
4. Comparison of the GH levels in medium containing growth hormone agonist analog to the GH levels in medium without the agonist



TABLE XXIX
IN VITRO GROWTH HORMONE RELEASE

<u>Dosage²</u>	<u>ΔGH¹</u>	<u>p Value⁴</u>
<u>H₂-His-D-Trp-Ala-Trp-D-Phe-Glu-NH₂</u>		
—	-251 + 105	---
1	-78 + 72	NS ³
3	-294 + 75	NS
10	-243 + 71	NS
30	117 + 41	<0.01

1. The mean of 9 assays given in terms of ng/ml incubation medium + standard error of the mean (SEM)
2. Given in terms of ng/ml incubation medium
3. NS denotes not significant
4. Comparison of the GH levels in medium containing growth hormone agonist analog to the GH levels in medium without the agonist

TABLE XXX

IN VITRO GROWTH HORMONE RELEASE

	Dosage ²	ΔGH^1	p Value ³
<u>H₂-His-D-Trp-Ala-Trp-D-Phe-HomoArg-NH₂</u>			
	---	-553 \pm 105	---
	1	-150 \pm 124	<0.02
	10	1,096 \pm 341	<0.001

up

1. The mean of 6 assays given in terms of ng/ml incubation medium + standard error of the mean (SEM)
2. Given in terms of ng/ml incubation medium
3. Comparison of the GH levels in medium containing growth hormone agonist analog to the GH levels in medium without the agonist



TABLE XXXI
IN VITRO GROWTH HORMONE RELEASE

<u>Dosage²</u>	<u>ΔGH¹</u>	<u>p-Value⁴</u>
<u>H₂-3-N-Me-D-Trp-Ala-Trp-D-Phe-Lys-NH₂</u>		
---	435 ± 176	---
1	165 ± 136	NS ³
10	1,857 ± 254	<0.01

1. The mean of 9 assays given in terms of ng/ml incubation medium ± standard error of the mean (SEM)
2. Given in terms of ng/ml incubation medium
3. NS denotes not significant
4. Comparison of the GH levels in medium containing growth hormone agonist analog to the GH levels in medium without the agonist



TABLE XXXII
IN VITRO GROWTH HORMONE RELEASE

<u>Dosage²</u>	<u>ΔGH¹</u>	<u>p-Value⁴</u>
<u>H₂-His-D-Trp-Ala-Trp-D-Phe-Lys-NHCH₂CH₃</u>		
---	435 \pm 176	---
1	726 \pm 115	NS ³
10	1,688 \pm 300	<0.01

1. The mean of 9 assays given in terms of ng/ml incubation medium \pm standard error of the mean (SEM)
2. Given in terms of ng/ml incubation medium
3. NS denotes not significant
4. Comparison of the GH levels in medium containing growth hormone agonist analog to the GH levels in medium without the agonist

TABLE XXXIII
IN VITRO GROWTH HORMONE RELEASE

Dosage ²	ΔGH^1	p Value ⁴
H ₂ -His-D-Trp-Ala-Trp-D-Phe-Orn-NH ₂		
---	-553 ± 105	---
1	-295 ± 93	NS ³
10	356 ± 92	<0.001

1. The mean of 6 assays given in terms of ng/ml incubation medium + standard error of the mean (SEM)
2. Given in terms of ng/ml incubation medium
3. NS denotes not significant
4. Comparison of the GH levels in medium containing growth hormone agonist analog to the GH levels in medium without the agonist

TABLE XXXIV

IN VITRO GROWTH HORMONE RELEASE

<u>Dosage²</u>	<u>ΔGH¹</u>	<u>p-Value³</u>
<u>H₂-His-D-Trp-Val-Trp-D-Phe-Lys-NH₂</u>		
---	-553 ± 105	---
30	-118 ± 65	<0.01

1. The mean of 6 assays given in terms of ng/ml incubation medium ± standard error of the mean (SEM)
2. Given in terms of ng/ml incubation medium
3. Comparison of the GH levels in medium containing growth hormone agonist analog to the GH levels in medium without the agonist



TABLE XXXV

IN VITRO GROWTH HORMONE RELEASE

Dosage ²	ΔGH ¹	p Value ⁴
H ₂ -His-D-Trp-Ser-Trp-D-Phe-Lys-NH ₂		
---	-125 ± 132	---
10	161 ± 157	NS ³
30	705 ± 149	<0.01

1. The mean of 6 assays given in terms of ng/ml incubation medium ± standard error of the mean (SEM)
2. Given in terms of ng/ml incubation medium
3. NS denotes not significant
4. Comparison of the GH levels in medium containing growth hormone agonist analog to the GH levels in medium without the agonist



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The results set forth in Tables VI-XXXV demonstrate that peptides within the scope of the instant invention can induce a significant in vitro release of growth hormone from the pituitary.

By introducing various other hormones, e.g., somatostatin, testosterone, cortisol, insulin, etc., into the incubation medium of Examples 11-30, one can study what effect these latter hormones have on the regulation of growth hormone secretion.

Example 61

In Vivo Growth Hormone Release Study

Female rats of the CD-1 strain were housed in a constant temperature room at 24°C. with 14 hours light and 10 hours darkness. The rats were fed Purina brand rat chow ad libitum. All studies were started between 0800 and 1000 hours.

Each female rat (21 days old; eight rats per dosage level shown in Table XVI) was intraperitoneally injected with a desired dosage of the peptide of Example 1. Approximately 15 minutes after injection, the rat was guillotined. A blood sample was collected from the guillotined rat. The blood sample was centrifuged and a serum sample was collected therefrom. Each serum sample was assayed for GH, in duplicate, by a standard radioimmunoassay (RIA). The mean of the GH values obtained per dosage level are set forth in Table XXXVI.

Example 62

In Vivo Growth Hormone Release Study

The procedure set forth in Example 61 was employed in an in vivo growth hormone release study of the peptide of Example 21 and the results therefrom are set forth in Table XXXVII.

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Example 63In Vivo Growth Hormone Release Study

The procedure set forth in Example 61 was employed in an in vivo growth hormone release study of the peptide of Example 22 and the results therefrom are set forth in Table XXXVIII.

Example 64In Vivo Growth Hormone Release Study

The procedure set forth in Example 61 was employed in an in vivo growth hormone release study of the peptide of Example 23 and the results therefrom are set forth in Table XXXIX.

Example 65In Vivo Growth Hormone Release Study

The procedure set forth in Example 61 was employed in an in vivo growth hormone release study of the peptide of Example 24 and the results therefrom are set forth in Table XXXX.

Example 66In Vivo Growth Hormone Release Study

The procedure set forth in Example 61 was employed in an in vivo growth hormone release study of the peptide of Example 25 and the results therefrom are set forth in Table XXXXI.

Example 67In Vivo Growth Hormone Release Study

The procedure set forth in Example 61 was employed in an in vivo growth hormone release study of the peptide of Example 26 and the results therefrom are set forth in Table XXXXII.



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Example 68In Vivo Growth Hormone Release Study

The procedure set forth in Example 61 was employed in an in vivo growth hormone release study of the peptide of Example 27 and the results therefrom are set forth in Table XXXXIII.

Example 69In Vivo Growth Hormone Release Study

The procedure set forth in Example 61 was employed in an in vivo growth hormone release study of the peptide of Example 16 and the results therefrom are set forth in Table XXXXIV.



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TABLE XXXVI
IN VIVO GROWTH HORMONE RELEASE

<u>Dosage²</u>	<u>ΔGH¹</u>	<u>p Value⁴</u>
<u>H₂-His-D-Trp-Ala-Trp-D-Phe-NH₂</u>		
Control	4 ± 1	---
0.1	1 ± 1	<0.05
1.0	2 ± 2	NS ³
10.0	2 ± 1	NS
100.0	82 ± 18	<0.001

1. The mean of 8 assays given in terms of ng/ml intraperitoneal + standard error of the mean (SEM)
2. Given in terms of μg/ml serum
3. NS denotes not significant
4. GH levels in serum of rats intraperitoneally injected with peptide compared to the GH levels in serum of control rats

TABLE XXXVII
IN VIVO GROWTH HORMONE RELEASE

<u>Dosage²</u>	<u>ΔGH¹</u>	<u>p Value³</u>
<u>H₂-His-D-Trp-Ala-Trp-D-Phe-Lys-OH</u>		
Control	1.7 ± 1	---
3.0	76 ± 24	<0.01
10.0	119 ± 25	<0.001
30.0	226 ± 43	<0.001

1. The mean of 8 assays given in terms of ng/ml subcutaneous medium ± standard error of the mean (SEM)
2. Given in terms of μg/ml serum
3. GH levels in serum of rats subcutaneously injected with peptide compared to the GH levels in serum of control rats

TABLE XXXVIII
IN VIVO GROWTH HORMONE RELEASE

<u>H₂-His-D-Trp-Ala-Trp-D-Phe-Arg-NH₂</u>	<u>Dosage²</u>	<u>AGH¹</u>	<u>p Value⁴</u>
Control		5.7 + 2.2	---
1.0		9.2 + 3.4	NS ³
3.0		32.2 + 5.5	<0.001
10.0		87.5 + 31	0.02
30.0		31.1 + 3.4	<0.001

1. The mean of 8 assays given in terms of ng/ml subcutaneous + standard error of the mean (SEM)
2. Given in terms of µg/ml serum
3. NS denotes not significant
4. GH levels in serum of rats subcutaneously injected with peptide compared to the GH levels in serum of control rats

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TABLE XXXIX
IN VIVO GROWTH HORMONE RELEASE

Dosage ²	AGH ¹	p value ⁴
<u>H₂-His-D-Trp-Ala-Trp-D-Phe-Gln-NH₂</u>		
Control	4.9 ± 0.9	---
1.0	1.4 ± 0.9	NS ³
3.0	3.8 ± 2.1	NS
10.0	32.1 ± 11.4	<0.05

1. The mean of 9 assays given in terms of ng/ml subcutaneous + standard error of the mean (SEM)
2. Given in terms of µg/ml serum
3. NS denotes not significant
4. GH levels in serum of rats subcutaneously injected with peptide compared to the GH levels in serum of control rats



TABLE XXXX
IN VIVO GROWTH HORMONE RELEASE

<u>Dosage²</u>	<u>ΔGH¹</u>	<u>p Value⁴</u>
<u>H₂-His-D-Trp-Ala-Trp-D-Phe-Glu-NH₂</u>		
Control	0.34 ± 0.19	---
1.0	0.10 ± 0	NS ³
3.0	1.50 ± 0.56	NS
10.0	1.4 ± 0.78	NS
30.0	8.06 ± 2.68	0.01

1. The assays given in terms of ng/ml subcutaneous ± standard error of the mean (SEM)
2. Given in terms of μg/ml serum
3. NS denotes not significant
4. GH levels in serum of rats subcutaneously injected with peptide compared to the GH levels in serum of control rats



TABLE XXXXI
IN VIVO GROWTH HORMONE RELEASE

<u>Dosage²</u>	<u>ΔGH¹</u>	<u>p-Value⁴</u>
<u>H₂-His-D-Trp-Ala-Trp-D-Phe-HomoArg-NH₂</u>		
Control	0 ± 0	---
0.3	3 ± 2	NS ³
10.0	62 ± 8	<0.001

1. The mean of 6 assays given in terms of ng/ml subcutaneous + standard error of the mean (SEM)
2. Given in terms of μg/ml serum
3. NS denotes not significant
4. GH levels in serum of rats subcutaneously injected with peptide compared to the GH levels in serum of control rats

TABLE XXXXII
IN VIVO GROWTH HORMONE RELEASE

<u>H₂-3-N-Me-His-D-Trp-Ala-Trp-D-Phe-Lys-NH₂</u>	<u>Dosage²</u>	<u>ΔGH¹</u>	<u>p-Value³</u>
Control		1.1 ± 0.6	---
0.3		45.0 ± 10	<0.001
10.0		128 ± 16	<0.001

1. The mean of 6 assays given in terms of ng/ml subcutaneous + standard error of the mean (SEM)
2. Given in terms of μg/ml serum
3. GH levels in serum of rats subcutaneously injected with peptide compared to the GH levels in serum of control rats



TABLE XXXXIII

IN VIVO GROWTH HORMONE RELEASE

<u>Dosage²</u>	<u>ΔGH¹</u>	<u>p Value³</u>
<u>H₂-His-D-Trp-Ala-Trp-D-Phe-Lys-NHCH₂CH₃</u>		
Control	1.1 ± 0.6	---
0.3	13.0 ± 5	<0.05
10.0	119 ± 12	<0.001

1. The mean of 6 assays given in terms of ng/ml subcutaneous + standard error of the mean (SEM)

2. Given in terms of μg/ml serum

3. GH levels in serum of rats subcutaneously injected with peptide compared to the GH levels in serum of control rats

TABLE XXXIV
IN VIVO GROWTH HORMONE RELEASE

<u>Dosage²</u>	<u>AGH¹</u>	<u>p Value⁴</u>
<u>H₂-His-D-Trp-Ala-Trp-D-Phe-Lys-NH₂</u>		
Control	0 ± 0	---
0.3	6 ± 4	NS ³
1.0	36 ± 15	<0.04
3.0	66 ± 15	<0.001
10.0	100 ± 19	<0.001
30.0	122 ± 4	<0.001

1. The mean of 6 assays given in terms of ng/ml subcutaneous + standard error of the mean (SEM)

2. Given in terms of µg/ml serum

3. NS denotes not significant

4. GH levels in serum of rats subcutaneously injected with peptide compared to the GH levels in serum of control rats



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The results set forth in Tables XXVI-XXXXIV demonstrate that some peptides within the scope of this invention can induce a significant in vivo release of growth hormone.

Example 70

In Vitro Growth Hormone Release Study

The procedure set forth in Example 31 was employed in an in vitro growth hormone release study of the growth promoting agent zeranol, $C_{18}H_{26}O_5$, and the results therefrom are set forth in Table XXXXV.

Example 71

In Vitro Growth Hormone Release Study

The procedure set forth in Example 31 was employed in an in vitro growth hormone release study of the peptide of Example 21 in combination with the growth promoting agent zeranol, $C_{18}H_{26}O_5$, and the results therefrom are set forth in Table XXXXVI. The results set forth in Table XXXXVI, when compared to the results set forth in Tables XXVI and XXXXV, demonstrate that a combination within the scope of the instant invention can induce a synergistic in vitro release of growth hormone from the pituitary.



TABLE XXXV
IN VITRO GROWTH HORMONE RELEASE

Dosage ²	AGH ¹	p Value ³
Zeranol; C ₁₈ H ₂₆ O ₅		
---	-115 ± 49	---
300	83 ± 63	0.02
3,000	87 ± 59	<0.02
30,000	683 ± 187	<0.001
100,000	-133 ± 98	NS ³

1. The mean of 9 assays given in terms of ng/ml incubation medium + standard error of the mean (SEM)
2. Given in terms of ng/ml incubation medium
3. NS denotes not significant
4. Comparison of the GH levels in medium containing growth hormone agonist analog to the GH levels in medium without the agonist

TABLE XXXXVI
IN VITRO GROWTH HORMONE RELEASE

Dosage ² Zeranol, C ₁₈ H ₂₆ O ₅ (A)	ΔGH ¹	p Value ³
H ₂ -His-D-Trp-Ala-Trp-D-Phe-Lys-NH ₂		
---	-309 + 39	---
3,000 A + 1B	2,183 + 399	<0.001
3,000 A + 3B	2,114 + 336	<0.001
30,000 A + 1B	2,266 + 327	<0.001
30,000 A + 3B	2,761 + 356	<0.001

1. The mean of 9 assays given in terms of ng/ml incubation medium + standard error of the mean (SEM)
2. Given in terms of ng/ml incubation medium
3. Comparison of the GH levels in medium containing growth hormone agonist analog to the GH levels in medium without the agonist



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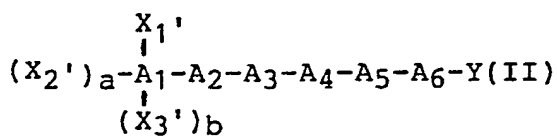
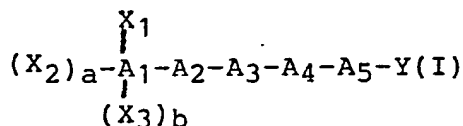
Based on this disclosure, many other modifications and ramifications will naturally suggest themselves to those skilled in the art. These are intended to be comprehended as within the scope of this invention.



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The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A peptide having a formula selected from a group consisting of



wherein

X_1 , X_2 , X_1' , X_2' , and X_3 are selected from a group consisting of N-terminal and desamino alpha-carbon substitutions;

a and b are 0 or 1, provided that a and b are 0 when A_1 is a desamino residue;

A_1 and A_4 are selected from a group consisting of His, Arg, Lys, α -Naphth, β -Naphth, Iql, Tyr, Trp, Phe, homologues and analogues thereof, and, with respect to A_1 , the desamino forms thereof;

A_2 and A_5 are selected from a group consisting of D-His, D-Arg, D-Lys, D- α -Naphth, D- β -Naphth, D-Iql, D-Tyr, D-Trp, D-Phe, homologues and analogues thereof, and, with respect to A_5 , the descarboxy forms thereof;

A_3 is selected from a group consisting of Gly, Ala, Val, Leu, Ile, Pro, Ser, Thr, Met, Asp, Glu, Asn, Gln, His, D-Ala, D-Val, D-Leu, D-Ile, D-Pro, D-Ser, D-Thr, D-Met, D-Asp, D-Glu, D-Asn, D-Gln, D-His, and homologues and analogues thereof;

A_6 is selected from a group consisting of amino acids of the L- and D- configuration, homologues and

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analogues thereof, and the descarboxy forms thereof; and

Y is selected from a group consisting of C-terminal and descarboxy alpha-carbon substitutions; and the pharmaceutically acceptable salts thereof;

provided that, when (1) a is 1 and b is 0 and X_1 and X_2 are selected from the group consisting of -H and -CH₃; (2) A_1 and A_4 are selected from the group consisting of Tyr, Trp, and Phe; (3) A_3 is selected from the group consisting of Gly, Ala, Val, Leu, Ile, Pro, Ser, Thr, Met, Asp, Glu, Asn, Gln, and His; and (4) Y is selected from the group consisting of -NR₁R₂, -CH₂OR, and -OR, wherein R, R₁, and R₂ are selected from a group consisting of hydrogen and straight and branched chain alkyl groups containing 1-6 carbon atoms; then at least one of A_2 and A_5 is selected such that it is not from the group consisting of D-Tyr, D-Trp, D-Phe, and, with respect to A_5 , the descarboxy forms thereof;

when (1) a is 1 and b is 0 and X_1 and X_2 are selected from the group consisting of -H and -CH₃; (2) A_2 and A_5 are selected from the group consisting of D-Tyr, D-Trp, D-Phe, and, with respect to A_5 , the descarboxy forms thereof; (3) A_3 is selected from the group consisting of Gly, Ala, Val, Leu, Ile, Pro, Ser, Thr, Met, Asp, Glu, Asn, Gln, and His; and (4) Y is selected from the group consisting of -NR₁R₂, -CH₂OR, and -OR, wherein R, R₁, R₂ are selected from a group consisting of hydrogen and straight and branched chain alkyl groups containing 1-6 carbon atoms; then at least one of A_1 and A_4 is selected such that it is not from a group consisting of Tyr, Trp, and Phe;

(1) when a is 1 and b is 0 and X_1' and X_2' are selected from the group consisting of -H, -CH₃, and



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-CHOCH₃; (2) A₁ and A₄ are selected from the group consisting of Tyr, Trp, and Phe; (3) A₃ is selected from the group consisting of Gly, Ala, Val, Leu, Ile, Ser, Thr, Met, Asn, and Gln; (4) A₆ is selected from the group consisting of Asn, Gln, Glu, Arg, Lys, Ser, Thr, and the descarboxy forms thereof; and (5) Y is selected from the group consisting of -NR₁R₂, -CH₂OR, and -OR, wherein R, R₁, and R₂ are selected from a group consisting of hydrogen and straight and branched chain alkyl groups containing 1-6 carbon atoms; then at least one of A₂ and A₅ is selected such that it is not from the group consisting of D-Tyr, D-Trp, and D-Phe; and

when (1) a is 1 and b is 0 and X₁' and X₂' are selected from the group consisting of -H, -CH₃ and PCHOCH₃; (2) A₂ and A₅ are selected from the group consisting of D-Tyr, D-Trp, and D-Phe; (3) A₃ is selected from the group consisting of Gly, Ala, Val, Leu, Ile, Ser, Thr, Met, Asn, and Gln; (4) A₆ is selected from the group consisting of Asn, Gln, Glu, Arg, Lys, Ser, Thr, and the descarboxy forms thereof; and (5) Y is selected from the group consisting of -NR₁R₂, -CH₂OR, and -OR, wherein R, R₁, R₂ are selected from a group consisting of hydrogen and straight and branched chain alkyl groups containing 1-6 carbon atoms; then at least one of A₁ and A₄ is selected such that it is not from a group consisting of Tyr, Trp, and Phe.

2. The peptide of claim 1 wherein:

a is 0 or 1 and b is 0;

X₁, X₂, X₁' and X₂' are selected from a group consisting of -R, -OR, and RC(O)-, wherein R is selected from a group consisting of hydrogen and straight and branched chain alkyl group containing 1-6 carbon atoms;



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A₁ and A₄ are selected from the group consisting of His, Tyr, Trp, Phe, homologues and analogues thereof, and, with respect to A₁, the desamino forms thereof;

A₂ and A₅ are selected from the group consisting of D-His, D-Tyr, D-Trp, D-Phe, homologues and analogues thereof, and, with respect to A₅, the descarboxy forms thereof;

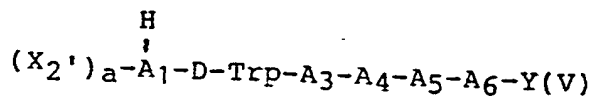
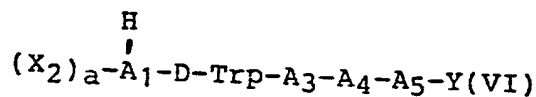
A₃ is selected from the group consisting of Gly, Ala, Ser, Asn, Pro, D-Ala, D-Ser, D-Asn, D-Pro, and homologues and analogues thereof;

A₆ is selected from the group consisting of Arg, Lys, Orn, His, Asp, Glu, Asn, Gln, D-Arg, D-Lys, D-Orn, D-His, D-Asp, D-Glu, D-Asn, D-Gln, D-Arg, homologues and analogues thereof, and descarboxy forms thereof;

Y is selected from the group consisting of -CH₂OH, -OR, and -NR₁R₂, wherein R, R₁ and R₂ are selected from the group consisting of hydrogen and straight or branched chain alkyl group containing 1-6 carbon atoms; and

the pharmaceutically acceptable salts thereof.

3. The peptide of claim 1 having the formula selected from the group consisting of



wherein

a is 0 or 1;

X₂ and X₂' are selected from the group consisting of RCO and R- wherein R is selected from the



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group consisting of hydrogen and alkyl groups containing 1-2 carbon atoms;

A₁ is selected from the group consisting of Tyr, O-Me-Tyr, His, 3-N-Me-His, p-Cl-Phe, and the desamino forms thereof;

A₃ is selected from the group consisting of Ala, Ser, and D-Ala;

A₄ is selected from the group consisting of Trp and Tyr;

A₅ is selected from the group consisting of D-Phe, D-His, D-Tyr, and D-p-Cl-Phe;

A₆ is selected from the group consisting of Arg, HomoArg, Lys, Orn, Asp, Glu, Asn, Gln, and D-Lys;

Y is selected from the group consisting of -OR, and -NHR, wherein R is selected from the group consisting of hydrogen and alkyl groups containing 1-2 carbon atoms; and

the pharmaceutically acceptable salts thereof.

4. The peptide of claim 1 of the formula selected from a group consisting of

H₂-His-D-Trp-Ala-Trp-D-Phe-NH₂,
 H₂-Tyr-D-Trp-Ala-Trp-D-His-NH₂,
 H₂-His-D-Trp-Ala-Trp-D-His-NH₂,
 H₂-His-D-Trp-Ala-Trp-D-Tyr-NH₂,
 H₂-Tyr-D-Trp-Ala-Trp-D-p-Cl-Phe-NH₂,
 H₂-p-Cl-Phe-D-Trp-Ala-Trp-D-Phe-NH₂,
 H-desaminoTyr-D-Trp-Ala-Trp-D-Phe-NH₂,

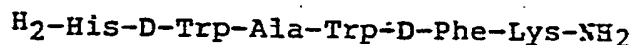
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CH₃CO-Tyr-D-Trp-Ala-Trp-D-Phe-NH₂,
 H₂-O-Me-Tyr-D-Trp-Ala-Trp-D-Phe-NH₂,
 H₂-Tyr-D-Trp-Ala-Trp-D-Phe-Met-NH₂,
 H₂-Tyr-D-Trp-Ala-Trp-D-Phe-Thr-NH₂,
 H₂-Tyr-D-Trp-Ala-Trp-D-Phe-Gln-OH,
 H₂-Tyr-D-Trp-Ala-Trp-D-Phe-Gln-NH₂,

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$\text{H}_2\text{-Tyr-D-Trp-Ala-Trp-D-Phe-Asn-NH}_2$,
 $\text{H}_2\text{-His-D-Trp-Ala-Trp-D-Phe-Lys-NH}_2$,
 $\text{H}_2\text{-Tyr-D-Trp-Ala-Trp-D-Phe-Lys-NH}_2$,
 $\text{H}_2\text{-Tyr-D-Trp-Ala-Trp-D-Phe-Glu-NH}_2$,
 $\text{H}_2\text{-Tyr-D-Trp-Ala-Trp-D-Phe-Phe-NH}_2$,
 $\text{H}_2\text{-Tyr-D-Trp-Ala-Trp-D-Phe-Gln-NH}_2$,
 $\text{H}_2\text{-His-D-Trp-Ala-Trp-D-Phe-Lys-CH}_3$,
 $\text{H}_2\text{-His-D-Trp-Ala-Trp-D-Phe-Arg-NH}_2$,
 $\text{H}_2\text{-His-D-Trp-Ala-Trp-D-Phe-Gln-NH}_2$,
 $\text{H}_2\text{-His-D-Trp-Ala-Trp-D-Phe-Glu-NH}_2$,
 $\text{H}_2\text{-His-D-Trp-Ala-Trp-D-Phe-HomoArg-NH}_2$,
 $\text{H}_2\text{-3-N-Me-His-D-Trp-Ala-Trp-D-Phe-Lys-NH}_2$,
 $\text{H}_2\text{-His-D-Trp-Ala-Trp-D-Phe-Lys-NHCH}_2\text{CH}_3$,
 $\text{H}_2\text{-His-D-Trp-Ala-Trp-D-Phe-Orn-NH}_2$,
 $\text{H}_2\text{-His-D-Trp-Val-Trp-D-Phe-Lys-NH}_2$, and
 $\text{H}_2\text{-His-D-Trp-Ser-Trp-D-Phe-Lys-NH}_2$.

5. A combination comprising:
 - (a) at least one growth promoting agent; and
 - (b) at least one peptide of any one of claims 1-3 or 4.
6. The combination of claim 5 wherein said peptide has the formula



and wherein said growth promoting agent is zeronol, $\text{C}_{18}\text{H}_{26}\text{O}$.

7. A method of releasing growth hormone from a pituitary comprising contacting said pituitary with the peptide of any one of claims 1-5 or 6.
8. A method of releasing growth hormone in vivo from a pituitary comprising contacting said pituitary with

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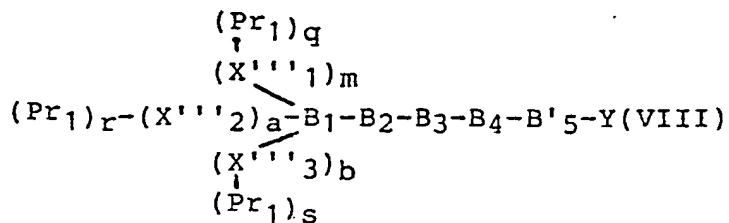
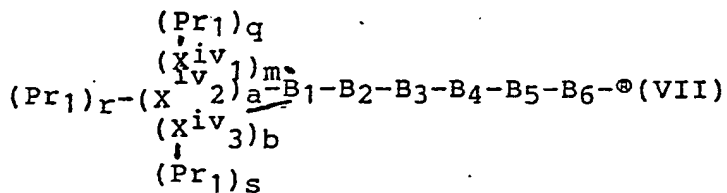
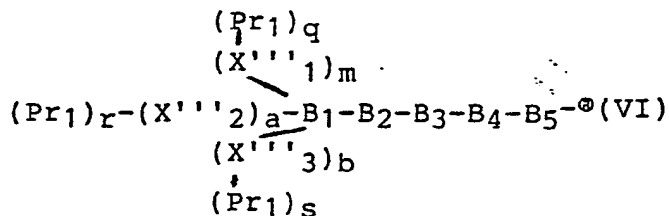
the peptide of claim 4 having a formula selected from the group consisting of

H₂-His-D-Trp-Ala-Trp-D-Phe-NH₂,
 H₂-His-D-Trp-Ala-Trp-D-Phe-Lys-NH₂,
 H₂-His-D-Trp-Ala-Trp-D-Phe-Lys-OH,
 H₂-His-D-Trp-Ala-Trp-D-Phe-Arg-NH₂,
 H₂-His-D-Trp-Ala-Trp-D-Phe-Gln-NH₂,
 H₂-His-D-Trp-Ala-Trp-D-Phe-Glu-NH₂,
 H₂-His-D-Trp-Ala-Trp-D-Phe-HomoArg-NH₂,
 H₂-3-N-Me-His-D-Trp-Ala-Trp-D-Phe-Lys-NH₂,

and

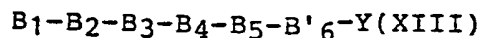
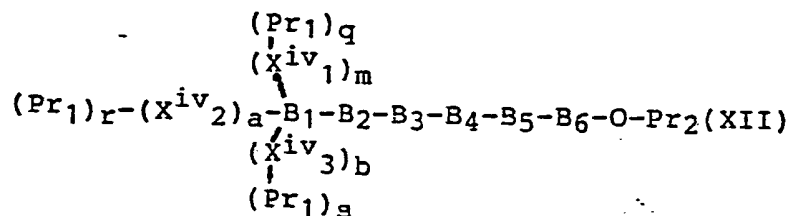
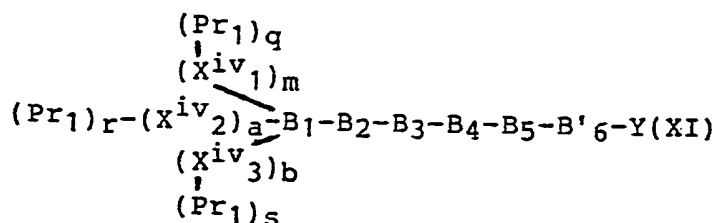
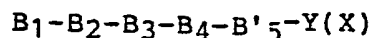
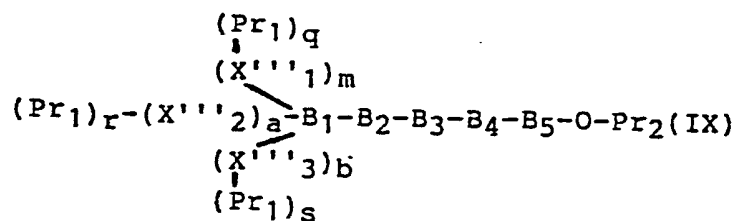
H₂-His-D-Trp-Ala-Trp-D-Phe-Lys-NHCH₂CH₃.

9. An intermediate composition having a formula selected from a group consisting of



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wherein

Pr_1 is an α -amino acid protecting group;

$a, b, m, q, r,$ and s are each 0 or 1;

$\text{X}'''_1, \text{X}'''_2, \text{X}'''_3, \text{X}^{\text{iv}}_2$ and X^{iv}_3 are selected from a group consisting of N-terminus and desamino alpha-carbon substitutions and radicals;

B_1 and B_4 are selected from a group consisting of His, Arg, Lys, α -Naphth, β -Naphth, Iql, Tyr, Trp, Phe, homologues and analogues thereof, the side-

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chain protected forms thereof, and, with respect to B_1 , the desamino forms thereof;

B_2 , B_5 , and B'_5 are selected from a group consisting of D-His, D-Arg, D-Lys, D- α -Naphth, D- β -Naphth, D-Iql, D-Tyr, D-Trp, D-Phe, homologues and analogues thereof, the side-chain protected forms thereof, and, with respect to B'_5 , the descarboxy forms thereof;

B_3 is selected from a group consisting of Gly, Ala, Val, Leu, Ile, Pro, Ser, Thr, Met, Asp, Glu, Asn, Gln, His, D-Ala, D-Val, D-Leu, D-Ile, D-Pro, D-Ser, D-Thr, D-Met, D-Asp, D-Glu, D-Asn, D-Gln, D-Arg, D-His, homologues and analogues thereof, and the side-chain protected forms thereof;

B_6 and B'_6 are selected from a group consisting of amino acids of the L- and D- configurations, homologues and analogues thereof, the side chain protected forms thereof, and, with respect to B'_6 the descarboxy forms thereof;

® is a resin;

Y is selected from a group consisting of C-terminal and descarboxy alpha-carbon substitutions; and

Pr_2 is a carboxyl protecting group;

provided that, when (1) a is 1 and b and m are 0 and X'''_2 is selected from the group consisting of -H and -CH₃; (2) B_1 and B_4 are selected from the group consisting of Tyr, Trp, Phe, and the side-chain protected forms thereof; (3) B_3 is selected

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from the group consisting of Gly, Ala, Val, Leu, Ile, Pro, Ser, Thr, Met, Asp, Glu, Asn, Gln, His, and the side-chain protected forms thereof; and, with respect to IV and VI, (4) Y is selected from the group consisting of $-NH_1R_2$, $-OR$, and $-CH_2OR$, wherein each R, R_1 , and R_2 is selected from a group consisting of hydrogen and straight and branched chain alkyl groups containing 1-6 carbon atoms; then at least one of B_2 , B_5 , or B'_5 is selected such that it is not from a group consisting of D-Tyr, D-Trp, D-Phe, and, with respect to B'_5 , the descarboxy forms thereof, and the side-chain protected forms thereof;

when (1) a is 1 and b and m are 0 and X'''_2 is selected from the group consisting of $-H$ and $-CH_3$; (2) B_2 and B_5 or B'_5 are selected from the group consisting of D-Tyr, D-Trp, D-Phe, and, with respect to B'_5 , the descarboxy forms thereof, and the side-chain protected forms thereof; (3) B_3 is selected from the group consisting of Gly, Ala, Val, Leu, Ile, Pro, Ser, Thr, Met, Asp, Glu, Asn, Gln, His and the side-chain protected forms thereof; and, with respect to IV and VI, (4) Y is selected from the group consisting of $-NR_1R_2$, $-OR$, and $-CH_2OR$, wherein each R, R_1 , and R_2 is selected from a group consisting of hydrogen and straight and branched chain alkyl groups containing 1-6 carbon atoms; then at least one of B_1 and B_4 is selected such that it is not from a group consisting of Tyr, Trp, Phe, and the side-chain protected forms thereof;

when (1) a is 1 and b and m are 0 and X^{iv}_2 is selected from the group consisting of $-H$, $-CH_3$, and $-CHOCH_3$; (2) B_1 and B_4 are selected from the group consisting of Tyr, Trp, Phe, and the side-chain protected forms thereof; (3) B_3 is selected from the



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group consisting of Gly, Ala, Val, Leu, Ile, Ser, Thr, Met, Asn, Gln, and the side-chain protected forms thereof; (4) B_6 or B'_6 is selected from the group consisting of Asn, Gln, Glu, Arg, Lys, Ser, Thr, and, with respect to B'_6 , the descarboxy forms thereof, and the side-chain protected forms thereof; and, with respect to XI and XIII, (5) Y is selected from the group consisting of $-NH_1R_2$, $-OR$, and $-CH_2OR$, wherein each R, R_1 , and R_2 is selected from a group consisting of hydrogen and straight and branched chain alkyl groups containing 1-6 carbon atoms; then at least one of B_2 or B_5 is selected such that it is not from a group consisting of D-Tyr, D-Trp, D-Phe, and the side-chain protected forms thereof; and

when (1) a is 1 and b and m are 0 and Xiv_2 is selected from the group consisting of $-H$, $-CH_3$, and $-CHOCH_3$; (2) B_2 and B_5 are selected from the group consisting of D-Tyr, D-Trp, D-Phe, and the side-chain protected forms thereof; (3) B_3 is selected from the group consisting of Gly, Ala, Val, Leu, Ile, Ser, Thr, Met, Asn, Gln, and the side-chain protected forms thereof; (4) B_6 or B'_6 is selected from the group consisting of Asn, Gln, Glu, Arg, Lys, Ser, Thr, and, with respect to B'_6 , the descarboxy forms thereof, and the side-chain protected forms thereof; and, with respect to XI and XIII, (5) Y is selected from the group consisting of PNR_1R_2 , $-OR$, and $-CH_2OR$, wherein each R, R_1 , and R_2 is selected from a group consisting of hydrogen and straight and branched chain alkyl groups containing 1-6 carbon atoms; then at least one of B_1 and B_4 is selected such that it is not from a group consisting of Tyr, Trp, Phe, and the side-chain protected forms thereof.

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10. The intermediate composition of claim 9 wherein:

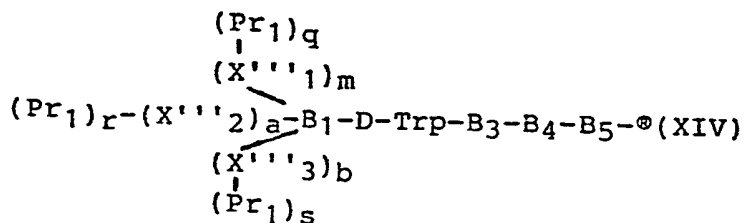
B₁ and B₄ are selected from the group consisting of His, Tyr, Trp, Phe, homologues and analogues thereof, and, with respect to B₁, the desamino forms thereof, and the side-chain protected forms thereof;

B₂, B₅, and B'₅ are selected from the group consisting of D-His, D-Tyr, D-Trp, D-Phe, homologues and analogues thereof, and, with respect to B'₅, the descarboxy forms thereof, and the side-chain protected forms thereof;

B₃ is selected from the group consisting of Gly, Ala, Ser, Asn, Pro, D-Ala, D-Ser, D-Asn, D-Pro, homologues and analogues thereof, and the side-chain protected forms thereof; and

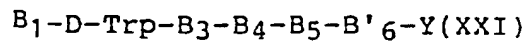
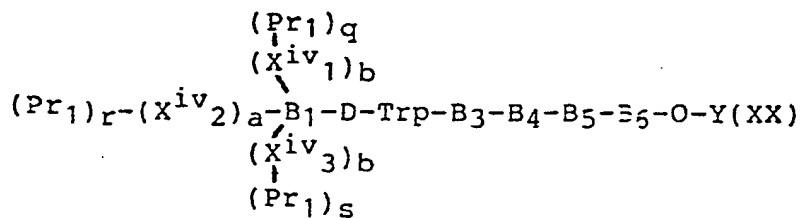
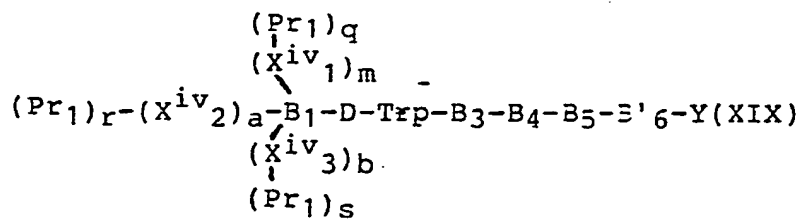
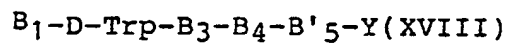
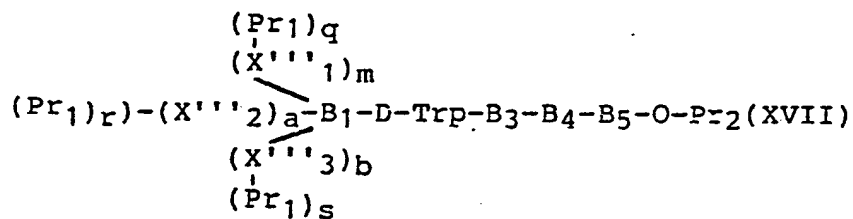
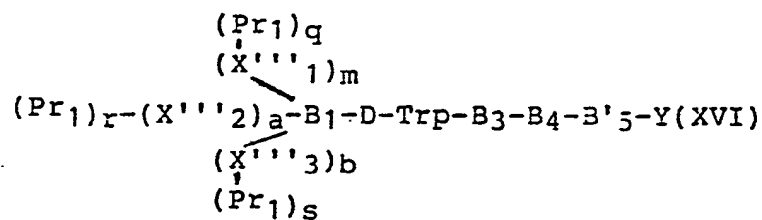
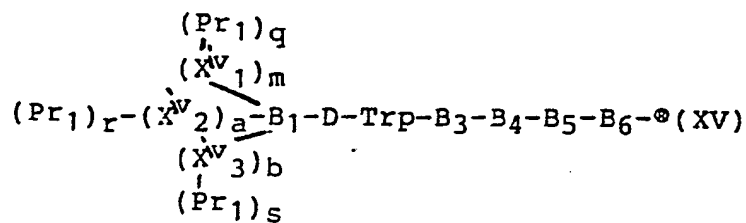
B₆ and B'₆ are selected from the group consisting of Arg, Lys, Orn, His, Asp, Glu, Asn, Gln, D-Arg, D-Lys, D-Orn, D-His, D-Asp, D-Glu, D-Asn, D-Gln, homologues and analogues thereof, and, with respect to B'₆, the descarboxy forms thereof, and the side-chain protected forms thereof.

11. The intermediate composition of claim 9 having the formula selected from the group consisting of



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wherein

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B₁ is selected from the group consisting of Tyr, O-Me-Tyr, His, 3-N-Me-His, p-Cl-Phe, the des-amino forms thereof, and the side-chain protected forms thereof;

B₃ is selected from the group consisting of Ala, Ser, D-Ala, and the side-chain protected forms thereof;

B₄ is selected from the group consisting of Trp, Tyr, and the side-chain protected forms thereof; and

B₅ and B'₅ are selected from the group consisting of D-Phe, D-His, D-Tyr, D-p-Cl-Phe, and, with respect to B'₅, the descarboxy forms thereof, and the side-chain protected forms thereof; and

B₆ and B'₆ are selected from the group consisting of Arg, HomoArg, Lys, Orn, Asp, Glu, Asn, Gln, D-Lys, the descarboxy forms thereof, and the side-chain protected forms thereof.

12. The intermediate composition of claim 9 of the formula selected from the group consisting of

Boc-His(Tos)-D-Trp-Ala-Trp-D-Phe-[®],
 Boc-Tyr(BrZ)-D-Trp-Ala-Trp-D-His(Tos)-[®],
 Boc-His(Tos)-D-Trp-Ala-Trp-D-His(Tos)-[®],
 Boc-His(Tos)-D-Trp-Ala-Trp-D-Tyr(BrZ)-[®],
 Boc-Tyr(BrZ)-D-Trp-Ala-Trp-D-p-Cl-Phe-[®],
 Boc-Tyr(BrZ)-D-Trp-Ala-Trp-D-Phe-[®],
 Boc-p-Cl-Phe-D-Trp-Ala-Trp-D-Phe-[®],
 3(p-OH-phenyl)propanoic acid-D-Trp-Ala-Trp-D-p-Cl-Phe-[®],
 Boc-O-Me-Tyr(BrZ)-D-Trp-Ala-Trp-D-Phe-[®],
 Boc-Tyr(BrZ)-D-Trp-Ala-Trp-D-Phe-Met-[®],
 BocOTyr(BrZ)-D-Trp-Ala-Trp-D-Phe-Thr(Bzl)-[®],
 Boc-Tyr(BrZ)-D-Trp-Ala-Trp-D-Phe-Glu-[®]

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benzyl ester-[⊗],
 Boc-Tyr(BrZ)-D-Trp-Ala-Trp-D-Phe-Gln-[⊗],
 Boc-Tyr(BrZ)-D-Trp-Ala-Trp-D-Phe-Asn-[⊗],
 Boc-His(Tos)-D-Trp-Ala-Trp-D-Phe-Lys(ClZ)-
[⊗],
 Boc-Tyr(BrZ)-D-Trp-Ala-Trp-D-His(Tos)-
 Lys(ClZ)-[⊗],
 Boc-Tyr(BrZ)-D-Trp-Ala-Trp-D-Phe-Glu-γ-
 Bzl-[⊗],
 Boc-Tyr(BrZ)-D-Trp-Ala-Trp-D-Phe-Phe-[⊗],
 Boc-Tyr(BrZ)-D-Trp-Gly-Trp-D-Phe-Gln-ONP-
[⊗],
 Boc-His(Tos)-D-Trp-Ala-Trp-D-Phe-Lys(ClZ)-
[⊗],
 Boc-His(Tos)-D-Trp-Ala-Trp-D-Phe-Arg(Tos)-
[⊗],
 Boc-His(Tos)-D-Trp-Ala-Trp-D-Phe-Gln-ONP-
[⊗],
 Boc-His(Tos)-D-Trp-Ala-Trp-D-Phe-Glu-γ-
 Bzl-[⊗],
 Boc-His(Tos)-D-Trp-Ala-Trp-D-Phe-
 HomoArg(Tos)-[⊗],
 Boc-3-N-Me-His-D-Trp-Ala-Trp-D-Phe-
 Lys(ClZ)-[⊗],
 Boc-His(Tos)-D-Trp-Ala-Trp-D-Phe-Lys(ClZ)-
[⊗],
 Boc-His(Tos)-D-Trp-Ala-Trp-D-Phe-Orn(Z)-[⊗],
 Boc-His(Tos)-D-Trp-Val-Trp-D-Phe-Lys(ClZ)-
[⊗], and
 Boc-His(Tos)-D-Trp-Ser(Bzl)-Trp-D-Phe-
 Lys(ClZ)-[⊗],

wherein

Boc is t-butyloxycarbonyl;

BrZ is o-bromobenzoyloxycarbonyl;



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BrZ is p-bromobenzyloxycarbonyl;
Bzl is benzyl;
γ-Bzl is γ-benzyl ester;
ClZ is o-chloro-benzyloxycarbonyl;
ONP is -nitrophenyl ester;
Tos is p-toluenesulfonyl;
Z is benzyloxycarbonyl;
⊙ is p-methylbenzhydralmine resin; and
⊙' is hydroxymethyl resin.

INTERNATIONAL SEARCH REPORT.

International Application No PCT/US 8 2 / 0 1 7 8 7

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) *		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC 3	007C 103/52	A61K 37/02
US	260 112.5R	424 177
II. FIELDS SEARCHED		
Minimum Documentation Searched *		
Classification System	Classification Symbols	
U.S.	260-112.5R 424-177	
Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched *		
III. DOCUMENTS CONSIDERED TO BE RELEVANT 14		
Category *	Citation of Document, 16 with indication, where appropriate, of the relevant passages 17	Relevant to Claim No. 18
X	US, A 4,228,158, (Momany) 14 October 1980	1 - 12
X	US, A 4,228,157 (Momany) 14 October 1980	1 - 12
A	US, A 4,228,156 (Momany) 14 October 1980	1 - 12
X	US, A 4,228,155 (Momany) 14 October 1980	1 - 12
X	US, A 4,226,857 (Momany) 07 October 1980	1 - 12
X	US, A 4,224,316 (Momany) 23 September 1980	1 - 12
X	US, A 4,223,021 (Momany) 16 September 1980	1 - 12
A	US, A 4,223,020 (Momany) 16 September 1980	1 - 12
X	US, A 4,223,019 (Momany) 16 September 1980	1 - 12
A	US, A 3,972,859 (Fujino) 03 August 1976	1 - 8
X	US, A 3,862,925 (Sarantakis) 28 January 1975	9 - 12
<p>* Special categories of cited documents: 15</p> <p>"A" document defining the general state of the art</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document cited for special reason other than those referred to in the other categories</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but on or after the priority date claimed</p> <p>"T" later document published on or after the international filing date or priority date and not in conflict with the application, but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search 1		Date of Mailing of this International Search Report 2
26 APR 1983		26 APR 1983
International Searching Authority 3		Signature of Authorized Officer 10
ISA/US		D. R. Phlips

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category*	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No 1 ¹⁸
A	US, A 3,664,925 (Sonenberg) 23 May 1972	1 - 8
A	US, A 3,803,309 (Desaulles) 09 April 1974	1 - 8
A	US, A 3,093,627 (Schwyzer) 11 June 1963	9 - 12
X	US, A 2,871,225 (Bortnick) 27 January 1959	9 - 12
X	EP, A 0,018,072 (Beckman Instruments, Inc.) 03 June 1980	1 - 12
A	N, J. Clinical Investigation, Vol. 65, 1980, Frohman et al., Partial Purification and Characterization of a Peptide with Growth Hormone-Releasing Activity from Extrapituitary Tumors in Patients with Acromegaly, pages 43-54.	9 - 12
A	N, Biochemical and Biophysical Research Communications, Vol. 45, 1971, Veber et al., Synthesis of a Proposed Growth Hormone Releasing Factor, pages 235-239	1 - 12
A	N, The Journal of Biological Chemistry, Vol. 246, No. 21, 1971, Schally et al., pages 6647-6650.	1 - 8
X	N, Journal of the American Chemical Society, December 30, 1970, Li et al., The Synthesis of a Protein Possessing Growth-Promoting and Lactogenic Activity, pages 7608-7609.	9 - 12
A	N, Journal of the American Chemical Society, Vol. 85, July 20, 1963, Merrifield, Solid Phase Peptide Synthesis. I. The Synthesis of a Tetrapeptide, pages 2149-2153.	9 - 12

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